Meningeal inflammation as a driver of cortical grey matter pathology and clinical progression in MS

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Key points

- The meninges are an immunologically active tissue barrier (blood-meningeal barrier/ blood-CSF barrier).
- The meninges represent an intrathecal immunological niche that compartmentalises chronic inflammation in multiple sclerosis (MS).
- Lymphoid neogenesis is an aberrant process occurring in the meninges of a substantial proportion (about 40%) of post-mortem progressive MS cases and diffuse meningeal inflammation is observed in the majority.
- Increased diffuse or compartmentalized meningeal inflammation is associated with a "surface-in" gradient of cortical cell pathology, including subpial demyelination and significant neuronal loss.
- Combined high levels of meningeal inflammation and subpial cortical damage are features of more severe and rapid disease progression.
- Meningeal inflammation represents a potential new therapeutic target to halt the disease progression.

Abstract

Recent evidence from the analysis of post-mortem MS brains, patient CSF samples and rodent models has suggested that the meninges play a key role in the inflammatory and neurodegenerative mechanisms underlying the pathology of progressive MS. The subarachnoid space and associated perivascular spaces are the access points for lymphocyte and monocyte/macrophage entry into the brain parenchyma and also the main route for the diffusion of inflammatory and cytotoxic molecules from the cerebrospinal fluid into the brain tissue. In addition, they act as an exit route for CNS-derived antigens, immune cells and metabolites. The reported close association between chronic meningeal inflammation and a more severe clinical course suggests that the build-up of immune cell aggregates in the meninges represents a rational target for therapeutic intervention. Therefore, it is vital to fully understand the precise cell and molecular mechanisms and the timing and anatomical features involved in the compartmentalisation of inflammation within the meningeal spaces in MS. We present a detailed review and discussion of the cellular, molecular and radiological evidence for a role for meningeal inflammation in MS and the clinical and therapeutic implications.

Introduction

Multiple sclerosis (MS) is a chronic disabling neurological condition affecting the central nervous system (CNS) and commonly becomes clinically evident during early adult life and occasionally during childhood. It is characterised in the majority of cases by bouts, or relapses, of neurological dysfunction, followed by complete or partial remission of symptoms. However, after a variable period of relapses and remissions, the majority of patients exhibit a clinically apparent progressive worsening of symptoms, which leads to a variable degree of physical and cognitive disability. Despite substantial advances in the development of disease-modifying therapeutics that effectively inhibit acute clinical relapses, there are currently no available treatments that are known to modify the underlying pathogenetic mechanisms that are responsible for the progressive component of MS[1]. This is in part due to the lack of a detailed understanding of the cellular and molecular mechanisms that lead to the accumulation of pathology in progressive MS. MS is characterised neuropathologically by the presence of focal areas of perivascular and meningeal inflammation, accompanied by demyelination, axonal degeneration, gliosis and blood-brain barrier changes in both the white and grey matter[2,3,4]. In addition to the focal pathology, diffuse changes are seen, which include widespread chronic microglial activation[5,6], axonal changes and neuron, axon and synapse loss in the grey matter (GM)[7,8,9,10].

Neuroimaging and post-mortem tissue analyses have focussed attention in recent years on the extensive pathology in the GM, particularly in the cerebral cortex[5,11,17,18], but also in other CNS regions[13,14,15,16]. Grey matter pathology is now thought to be responsible for many of the complex neurological manifestations associated with disease progression, not only restricted to motor and sensory symptoms but also including disruption of cognition, mood and increased risk of seizures. One of the intriguing pathological features of the GM in a large proportion of MS brains examined at post-mortem is the relationship between the often extensive demyelination of the sub-pial layers of the cerebral cortex[5,11,17,18] ,and the presence of substantial immune cell infiltrates in the overlying meninges[19,20,21,22]. The subpial demyelination is a pathological feature that is MS-specific and is not evident in other neurological and inflammatory diseases[18,23]. When MS was compared with a large cohort of autoimmune, inflammatory and neoplastic diseases of the central nervous system (CNS) with potentially predominant immune cell infiltration of the meninges and upper cortical layers, including acute disseminated encephalomyelitis (ADEM), neuromyelitis optica (NMO), viral and bacterial meningoencephalitis, progressive multifocal leukoencephalopathy (PML), subacute sclerosing panencephalitis (SSPE), carcinomatous and lymphomatous meningitis, extensive ribbon-like subpial demyelination and follicle-like structures were only observed in MS[18]. The close alignment of the cortical pathology with the cerebrospinal fluid (CSF) filled space of the sub-arachnoid meninges suggests that tissue damage may arise from the diffusion of cytotoxic soluble factors produced by immune cells across the pia mater and glia limitans[3,5]. In addition to diffuse immune cell infiltration, ectopic lymphoid-like aggregates develop in the leptomeninges of the deep cortical sulci in a substantial proportion of secondary progressive MS brains and their presence correlates with the clinical severity and rate of disease progression[19,20,21,22]. In vivo correlations between the levels of certain cytokines and chemokines in the CSF of MS patients and the degree of cortical pathology seen on MRI at early stages of the disease course[25,26], support several findings (**Table 1**) suggesting that the meningeal inflammation may be playing an important role in driving or exacerbating the pathological substrates of accumulating disability.

Here we review and discuss what is known about the structural, cellular and molecular composition of the meninges and how this changes in MS; the development and maintenance of lymphoid-like tissues in response to chronic inflammation; the relationship with the neuropathology of the underlying brain parenchyma; in vivo monitoring of meningeal inflammation; and the clinical and therapeutic implications of this organised compartmentalised inflammation for progression in MS.

The Meninges as an immune interface

Structure of the meninges

The meninges are highly vascularized tissues composed of the dura, the closely apposed arachnoid layer and the pia mater, which overlies the surface of the brain and spinal cord parenchyma, where it interacts with glial-derived basement membranes and astrocyte end-feet. The fibrous dura contains large arteries and veins and the draining lymphatic network, through which constituents and cells of the CSF can reach the lymph[27,28]. However, the exact route, where and how CSF components may pass the barrier of the arachnoid to reach lymphatic vessels of the dura remain still unclear. The arachnoid mater partitions the underlying SAS from the dura and contains a meshwork of trabeculae or tissue struts, which traverse and partly divide this important site of molecular communication and distribution. Arteries and veins of the SAS are suspended and either fully (arteries) or partly (veins) ensheathed by the squamous epithelial-like cells of the pia mater[29]. The cells of the pia are connected by gap junctions and desmosomes but lack the tight junction assemblies found in the arachnoid layer. Therefore, the pia represents a semi-permeable barrier separating the cerebrovasculature and SAS from the glial limitans and the perivascular spaces. Arteries extend into the brain wrapped with a continuous pia and with very tightly apposed arterial and glial basement membranes. Cerebrospinal fluid is continuous between these spaces, meaning that subpial and perivenular tissues are exposed to many of the same factors that can affect the cells of the parenchyma. Branching arterioles lose their complete pial-ensheathment and perivascular spaces can be seen at the level of the post-capillary venules and cortical veins, which have a fenestrated pial covering[17, 18,29,30,31,32]. The SAS and perivascular spaces are thus a continuous compartment. Together, the arachnoid and pia constitute the leptomeninges, which is an important site of immune monitoring[30,33].

The meninges as an immunologically active tissue barrier

The CSF-filled meninges are an important immune interface between the peripheral circulation and the brain and spinal cord parenchyma and an ideal site for immune surveillance, as they act as a barrier to the entry of foreign infectious agents into the brain. In addition, the composition of the CSF in the SAS reflects the physiological and pathological state of the underlying brain and allows signalling from the brain to the immune system via the perivascular spaces and pial surface. Migrating memory T-cells, even those targeting irrelevant antigens such as ovalbumin, can cross the blood-CSF barrier into the SAS[34]. Once they have crossed the barrier formed by the cerebroendothelial cells, infiltrating lymphocytes adhere to the trabeculae and crawl in an intermittent pattern or may be carried by the flow of the circulating CSF. As in other tissues, B- and T-cell extravasation is VLA-4/α-4 integrin-dependent and this is supported by earlier clinical studies demonstrating a reduction in intrathecal T, B and plasma-cell density, and the frequency of oligoclonal band positive individuals in natalizumab treated patients at two years[35,36,37]. Another CAM, activated leukocyte cell adhesion molecule (ALCAM), has also been demonstrated to be important for immune cell trafficking and is particularly involved in B-cell extravasation to the leptomeninges and the development of disease in a murine EAE model induced by injection of recombinant MOG38[38].

Experimental observations in murine EAE models using intra-vital imaging, single-cell cytometry and RNA sequencing, implicate the leptomeninges as a staging-post in T-cell infiltration and can prevent T-effector cell entry into CNS tissue in the absence of suitable re-stimulation by specialist resident APCs[39,40]. Infiltrating T lymphocytes make passing contacts with APCs, but can only cross the second CSF-to-brain barrier at sites of injury or inflammation, and following re-stimulation with cognate ligand[41]. The leptomeningeal and perivascular spaces contain macrophages that are long lived and self-renewing, that circulate within the CSF-filled territories and are seen as vital for normal CNS homeostasis, defence and actively participate in immune activation and autoimmunity[42]. Although MHC class II^+ macrophages represent the main population of cells in the meninges with antigen-presenting capability, very small numbers of DC-SIGN/CD209 expressing DCs have also been found in the perivascular spaces of the normal human brain, but were only very rarely seen in the meninges[43,44]. Single cell mapping approaches in the murine CNS revealed the presence of predominantly MHC class II^+ Lyve-1^+ border associated macrophages in the normal meninges, together with small numbers of 3 distinct subsets of bone marrow derived DCs[42]. However, similar subsets of DCs have yet to be identified in the human CNS. In addition to border macrophages and classical DCs, MHC class-II^+ B-lymphocytes are also able to act as APCs for brain homing effector memory T-lymphocytes[45], although very few B-lymphocytes are found in the human meninges in the non-diseased state[21].

T and B-lymphocytes that access the subarachnoid or perivascular spaces are likely to indirectly impact the brain and cord parenchyma through secreted cytokines, which change the composition of the CSF, modulate leptomeningeal and epithelial cells and in experimental approaches in rats have been shown to affect underlying tissues through the semi-permeable nature of the pial and glial basement membranes[95,96]. Small molecule tracers <40kDa, which would include most inflammatory cytokines, can be found in the interstitia following infusion into the rodent SAS, for example[46,41,47,48]. Analysis of MS CSF has revealed much about this active inflammatory process at all stages of the disease and supports evidence for the compartmentalisation and effector function of pathologically relevant lymphocytes in these territories, which may underlie the ineffectiveness of treatments in the majority of patients with long-standing disease[25,26].

The cellular nature of the inflammation in the meninges in MS

The presence of immune cell infiltrates in the meninges is a characteristic feature of MS pathology, although the degree of infiltration is extremely heterogeneous, ranging from a few cells to larger but diffuse infiltrates, and finally very large lymphoid-like aggregates[21]. Substantial infiltrates have been found in the meninges of cortical biopsies from very early MS cases[24], in post-mortem brains from very short disease duration acute cases[49], as well as both SPMS and PPMS brain[11,19,20,21,50] (Fig 1-2). Although these immune cell infiltrates have recently attracted much attention, the presence of large dense aggregates of leptomeningeal infiltrating cells were reported in a number of much earlier studies[51,52,53], which generally noted that the extent of infiltration in some extreme cases approximated that seen in meningitis. Although they were most frequently found in the cortical sulci, similar dense immune cell aggregates were also identified in the meninges of the spinal cord[11] and in narrow deep infoldings of the cerebellar cortex[15]. A few studies have been unable to identify these large B-cell rich immune cell aggregates in post-mortem MS brains [44,110, 107,18], but this may have been due to limited sampling and the use of different technical procedures when preparing the MS brain tissues that results in loss of the meninges or the cells within the meninges[54] .

Diffuse infiltrates in the MS meninges comprise CD4+ and CD8+ T lymphocytes and CD19/20+ B-lymphocytes, with an approximate ratio for T:B cells of between 2:1 to 3:1 and for CD8:CD4 T-cells

of 2:1[6,11,19,20,21,54,55]. Further phenotyping of these cells identified CD8+CD161+ IFNγ expressing effector memory T cells[56], CD8+CD57+ effector T-cells[57] and LTα expressing CD3+ T-cells[58]. Highly variable numbers of CD138+/Ig+ plasmablasts and plasma cells and MHC-II+ macrophages/monocytes are also found diffusely distributed along the meninges[55, 63].

More extensive characterisation of the cellular components and organisation of tertiary lymphoid-like dense infiltrates revealed that between 32-50% of post-mortem SPMS brains exhibit the presence of lymphoid-like immune cell aggregates in the cortical meninges[20,21,59] (Figure 3). A further 15-22% of SPMS post-mortem brains contained large meningeal immune cell aggregates without lymphoid-like organisation[19,20], whilst the rest of the cases had either very few meningeal immune cells or larger numbers but diffusely distributed[21]. These findings all suggest that meningeal lymphoid-like infiltrates are a common feature of the progressive MS brain, but exhibit a large degree of heterogeneity and can be found at all stages of development[60]. The lymphoid-like infiltrates are characterised by the presence of dense aggregates of CD19/20+ B-cells of varying size, with some filling the entire sulcus[6,19,20,21]. Varying proportions of these B-cells express the proliferation marker Ki67, suggesting the occurrence of antigen presentation and clonal expansion within the aggregates[19,20]. Both CD4+ and CD8+ T-cells are more diffusely distributed, thus giving rise to separate T- and B-cell domains in the most organised infiltrates. CD4+ T-follicular helper cells expressing CXCR5 and NFATc1 and CD4+CD69+ tissue resident cells could be demonstrated, but FoxP3+ T-follicular regulatory cells were absent[59]. Networks of CD21+, CD35+ and CXCL13+ processes of FDCs are seen to be in intimate contact with CD20+ B-cells. A small number of the largest and most organised lymphoid-like infiltrates contained cells expressing activation induced cytidine deaminase (AICD) and cells expressed the anti-apoptotic BCL-2, indicating germinal centre formation[61]. Clonal analysis of the B-cell aggregates identified clones that were shared between the meninges and perivascular infiltrates of white matter lesions, with a relative clonal expansion of 24% in the meninges and a 90% use of an IgG isotype, indicating their antigen experienced phenotype[62]. CD138+ Ig+ plasma cells were present in most lymphoid-like aggregates, often in a parafollicular distribution[5,21,59,63].

Lymphoid tissue neogenesis in MS in comparison to other chronic inflammatory diseases

The observation that the immune cell aggregates in the MS meninges appear to be at various stages of development is entirely in keeping with observations from a large number of non-CNS chronic inflammatory conditions in which lymphoid-like infiltrates have been identified and characterised. Chronic inflammation is a characteristic of many human disease states involving non-CNS tissues and organs and it has been demonstrated that in many of these disorders the immune cell infiltrate becomes increasingly organised into distinct ectopic lymphoid tissues/organs[64,65] or TLOs through the aberrant expression of lymphoid homing chemokines. Ectopic lymphoid tissues at various stages of development have been observed in non-resolving autoimmune conditions (rheumatoid arthritis, psoriatic arthritis, Hashimoto's thyroiditis, Grave's disease, myasthenia gravis, Sjogren's syndrome)[66,67,68,69,70,71], transplant rejection[72,73], chronic bacterial and viral infections (Helicobacter pylori induced gastritis, hepatitis-C, Lyme disease)[74,75,76], cancers (lung carcinoma; melanoma)[77,78], and idiopathic lung and circulatory diseases (pulmonary arterial hypertension, chronic obstructive pulmonary disease, pulmonary fibrosis, obliterative bronchiolitis, atherosclerosis)[58,79,80,81,82]. In all these conditions, they only develop in a subset of cases and also exhibit a highly variable level of organisation and frequency. This is also the case in MS where lymphoid structures with varying levels of sophistication have been identified in the meninges of up to 62% of cases[21], which is remarkably

similar to the frequency of TLO formation in the synovial tissues of rheumatoid arthritis and psoriatic arthritis patients[67,68].

In all these conditions involving chronic inflammation, the presence of TLOs is associated with more severe disease[64] and this is also the case in MS[21]. It is thought that these complex lymphoid structures develop in a tissue relevant context in response to an increased need for a localised immune response. In the presence of compartmentalised chronic inflammation, it is likely that they result from the induction of specific inflammatory mediators, in particular lymphoid homing chemokines, that are known to be involved in "lymphoid neogenesis" during development, such as TNF, LTα/β and CXCL13. While it has been difficult to establish a direct link between TLO formation and pathological tissue damage, it is clear that the continued presence of pro-inflammatory and cytotoxic cytokines and chemokines, and autoantibodies, is unlikely to be anything other than deleterious, with the possible exception of infectious diseases.

Ectopic TLOs in non-CNS tissues have been shown to contain memory CD4+ and CD8+ T cells, naive and memory CD19/CD20+ B cells, IL21+PD1+ T follicular helper cells (Tfh), CD11c+ dendritic cells, fibroblastic reticular cells, CD35+CXCL13+ follicular dendritic cells and CD138+ plasma cells[67,68,80]. In addition, B-cell follicles with germinal centres, high endothelial venules (HEVs) and lymphatic vessels are present in some cases. Those tissues that are very highly infiltrated often develop the most organised lymphoid structures and it is likely that the particular tissue environment influences the degree of organisation. For example, the TLOs that develop in solid tissues, such as in the lung, often develop more organisation than those that develop in a fluid filled space, as in RA and MS[67,80]. In MS the lymphoid-like tissues develop in the CSF filled subarachnoid space and rarely exhibit HEVs and lymphatic channels[19].

Mechanisms of initiation and maintenance of compartmentalized inflammation in the meninges

As discussed earlier, there is substantial evidence that the meninges are one of the earliest sites of antigen recognition and presentation with respect to inflammation in the nervous system, and animal model studies have provided evidence concerning the mechanisms involved[39,40,41,42]. In contrast, little is known concerning the cellular and molecular events that initiate and maintain chronic compartmentalised inflammation in the meninges in progressive MS. The observation in non-CNS tissues, as well as the MS meninges, that the most well developed tertiary lymphoid-like structures occur in highly inflamed tissues, suggests that extensive activation of local immune cells is required to initiate their formation[64,65]. Not unsurprisingly, TLO formation involves many of the same molecular pathways involved in lymphoid organogenesis during early development. Experimental studies in mice of other chronic inflammatory diseases, such as rheumatoid arthritis and autoimmune gastritis[84,85], indicate that stromal cells (including fibroblasts), VSMCs, pericytes, epithelial cells, blood and lymphatic endothelial cells, can be stimulated by LTα to play a key role in TLO formation, due to their expression of lymphoid cytokines and chemokines[84,86,87,88,89]. Under chronic inflammatory conditions, in response to LTα/β interaction with the LTβ receptor, stromal fibroblast cells undergo complex phenotypical changes and acquire lymphoid tissue organizer (LTo)-like cells features in order to release lymphorganogenic chemokines, such as CXCL13, CXCL12, CCL19 and CCL21, and organize local immune responses by secreting lymphoid chemokines and upregulating integrins, such as vascular cell adhesion molecule-1 and intercellular cell adhesion molecule-1, in order to favour the recruitment of immune cells to the local chronic inflammatory site[87,90,91]. CCL19 and 21 interaction with CCR7 on activated lymphocytes then controls the organisation of T-cell

zones, whilst CXCL13 acting on the CXCR5 receptor is required for the attraction and organisation of B-cells[74,84,86,88] (Fig. 2).

Recent post-mortem MS tissue and experimental animal studies provide a number of indicators towards the mechanisms involved in the development of TLOs in the MS meninges. Analysis of meningeal tissues and CSF from MS brains with lymphoid-like immune cell infiltrates identified the presence of elevated gene and protein expression of cytokines and chemokines involved in lympho-organogenesis and sustained B-cell activity, including TNF, LTα, CXCL10, CXCL13, IL6 and IL10[25]. Increases in the same soluble mediators were found in the CSF of drug naive MS patients who had elevated levels of cortical grey matter pathology on MRI at diagnosis[25]. Myeloid cells and CD3+ T-cells in the post-mortem MS meninges have been shown to express TNF and LTα respectively[5,58,92], whereas CXCL13-expressing stromal cells form FDC networks[19,20]. Expression of the CXCL10 chemokine is elevated in MS CSF and CXCR3-expressing B- and T-cells are enriched in CSF, meninges and brain in comparison to blood[93]. The T-cell attractant chemokines CCL19 and 21 have recently been shown to be elevated in the post-mortem CSF of MS brains harbouring lymphoid-like meningeal infiltrates[94] (Fig 2). Thus, all the necessary molecules required to initiate and maintain the presence of ectopic lymphoid tissues can be demonstrated to be present in the MS meninges and CSF.

Persistent ectopic expression of TNF or LTα in the meningeal space in rat model has been demonstrated to produce both extensive diffuse and organised immune cell infiltration and in the case of LTα gives rise to tertiary lymphoid like structures highly reminiscent of the MS meninges[95,96] (Fig 2). The meningeal immune cell infiltration was associated with accumulating neuron loss in the underlying cortical GM. Sub-pial cortical demyelination was also observed, but was only substantial when the animals had been pre-immunised with a low dose of recombinant MOG protein[58]. These results suggest that persistent expression of pro-inflammatory cytokines involved in lymphoid organogenesis in the meningeal space could be responsible for much of the pathology seen in MS. Meningeal inflammation accompanied by various stages of TLO formation has also been reported in several autoimmune encephalitis based murine models of MS, including relapsing remitting EAE in the SJL/J mouse immunised with PLP peptide[94], in chronic progressive EAE in the Biozzi-ABH mouse[94] and in C57Bl/6 mice immunised with an MBP-PLP fusion protein[97] . Adoptive transfer of MOG-specific Th-17 T-cells into C57Bl/6 mice[98] and PLP immunised SJL/J mice[99] also induced ectopic lymphoid follicles in the spinal cord, suggesting a role for CNS targeted Th17 cells in the induction of FDCs from meningeal stromal cells. However, the molecular mechanisms involved in these animal models and their relevance to MS itself requires further investigation.

EBV- pathological driver, incidental finding or valuable biomarker?

Accumulating epidemiological studies together with neuropathological and gene expression analyses of immune cells invading the MS CSF and brain parenchyma support the hypothesis that in situ deregulation of Epstein-Barr virus (EBV), a B cell tropic virus, and EBV-induced immunopathology might play a key role in CNS damage in MS[100,101,133]. Meningeal lymphoid structures and perivascular immune infiltrates, enriched in B cells, have been suggested as the main CNS sites of EBV persistence, substantiating a direct link between EBV infection and B cell dysregulation in MS[61,102,103]. Antiviral responses, and in particular type I interferon release, are strong inducers of CXCL13 expression, driving CXCR5-dependent recruitment of B cells and formation of ectopic germinal centres[104]. This evidence suggests the possibility that continued reactivation of T cells by immortalized EBV infected B cells in the subarachnoid space could then promote damage in the

adjacent grey matter cortical tissues. High-affinity molecular mimicry between the EBV transcription factor EBV nuclear antigen 1 (EBNA1) and the central nervous system protein glial cell adhesion molecule (GlialCAM) might represent the potential mechanistic link for the association between MS and EBV, as revealed also by the high levels of anti-EBNA1 and anti-GlialCAM antibodies in MS CSF [105]. The presence of EBV proteins in B-cells in MS tissue has been validated by some[102-104,105,133], but not all[106,107, 110] recent studies, suggesting that further detailed assessment of the cell and molecular mechanisms and alterations directly linked to EBV–host interactions are necessary in order to understand whether such infection has MS-specific causative functions or is linked to persisting chronic inflammatory CNS conditions.

The relationship of meningeal inflammation to cortical pathology

Cortical demyelination

Demyelination of the cortical GM has been demonstrated in about 90% of patients with chronic MS in post-mortem tissue studies, with up to 70% of the cortical area demyelinated in a proportion of patients[7,11,14,21]. This constitutes a much greater area than WM demyelination and thus is highly likely to contribute to neuronal and synaptic dysfunction/loss and to accumulation of neurological deficits and disease progression in MS patients[2,108]. In particular, ribbon-like subpial demyelination, comprising the largest fraction (65% or more) of cortical lesions, is uniquely seen in the MS brain and is not observed in other CNS inflammatory or neoplastic diseases with potential involvement of the meninges and upper cortical layers[17,18,119]. Cortical demyelinated lesions themselves display a relative paucity of inflammatory cell infiltrates and BBB alterations when compared to the subcortical white matter ones[7,17]. However, as described extensively above, in the vast majority of studies carried out on post-mortem human brain tissues, sub-pial demyelination appears closely associated with increased immune cell infiltration in the overlying meninges, either as lymphoid-like aggregates or as increased diffuse infiltrates [5,6,11,15,19-22,24,49,53]. One study failed to find an association[44], but only limited sampling was employed and the preservation of the structure and cellular content of the meninges may not have been optimal[54]. Whether there is an absolute association between sub-pial lesions and meningeal infiltrates has yet to be analysed and it remains possible that the lesions are more closely related to the general composition of the CSF than the immediate cellular apposition. Whether the meningeal immune cell aggregates and the underlying cortical lesions seen in the MS brain are dynamic in nature cannot currently be resolved.

One of the main characteristics of demyelinating cortical pathology, in particular in active progressive MS, is the presence of extensive microglial activation, identified by increased cell numbers and complexity of processes[5,6,110], expression of pro-inflammatory markers such as TNF[111,112], inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO), β-macroglobulin, CD68, MHC-class II, allograft inflammatory factor-1 and HMGB1[54,113]. Cortical and deep grey matter microglia can express either an anti-inflammatory phenotype to promote survival, i.e by synaptic stripping, or repair by the release of growth promoting and myelin repair factors such as CD163+ and Siglec-11[54], or proinflammatory functions as suggested from observations of synaptic, axo-glial and striatal degeneration in an environment of acute inflammation involving polymorphonucleocytes and activated microglia [9,114,115,116,117,118,119].Many of these studies were performed in models and need to be validated in MS tissues to have relevance to MS itself. More recently, two microglial phenotypes have been described in the MS cortex, an MS1 population with increased expression of the activation markers HLA class II and CD68, closely apposed to neuronal cell bodies and associated with relative neuronal sparing, and an MS2 population with decreased P2Y12 and TMEM119 expression that were

associated with increased neuronal loss and an increased presence of B cells in the adjacent meninges[6]. In addition, HLA-DRB1*15 status is associated with modulation of the relationship between microglial inflammation and synaptic neuronal alteration in MS[120]. Recent transcriptomic analysis in WM and GM of post-mortem MS and control brains, confirms the heterogeneity of microglial phenotypes in GM compared to WM, suggesting higher expression of several genes, such as STAT2 and IRF9, involved in modulation of the type-I IFN response[121]. However, the relationship between distant inflammatory events and neuronal control of microglial reactivity and function in the tissue still needs to be fully explored, as well as the contribution of the local neuronal population to cellular homeostasis through the release or signalling or microglial regulators, for example CD200/R and CX3CL/R interactions.

The role of GM astrocytes in MS cortical pathology has been little investigated. Their distribution and extensive interactions within multiple cell layers[122], their regional heterogeneity and their vital role in formation of the surface glial limitans and barrier formation around parenchymal vessels, suggests that they are likely to play important homeostatic functions. Loss of gap junction connectivity and consequent oligodendrocyte degeneration may be a crucial mediator of cortical pathology[123,124]. Cortical astrocytes display evidence of change in MS grey matter lesions, but this is much less than the hypertrophy seen in chronic white matter lesions. A surface-in gradient of substantial astrocyte loss was detected in the most external cortical layers of subpial cortical MS lesions associated with elevated meningeal infiltration[5] . Primary astrocyte loss is now recognised as key to oligodendrocyte and myelin damage in NMO and was suggested as a possible cause of pattern III demyelination and oligodendrocyte apoptosis in acute fulminant MS[125]. These findings support the assertion that a selective loss of astrocytes is a feature of MS, which may corroborate the finding of raised CSF GFAP in cases of active disease[126,127]. Astrocytes may also be involved in disease induction via production of molecular mediators, such as ROS/RNS, glutamate, ATP, IL1, TNF, IL6, IL12, and complement, all of which can have potentially direct toxic effects on neurons/axons and oligodendrocytes/myelin[128]. Astrocyte overexpression of BAFF (B cell activator factor) may promote the survival of BAFF-R–expressing B cells, and of CXCL12 expression, which mediates germinal centre reactions in MS brain, and may have a key role in the persistence and clonal expansion of B cells in MS CNS[124,129,130,131]. Further in-depth investigations of astrocyte functions in new and acute subpial inflammatory GM lesions are required to understand their fate and role in the disease processes.

Very few infiltrating lymphocytes and or perivascular B cells or antibody secreting plasma cells have been detected in chronic MS cortical lesions[17,132,133], although the presence and degree of sub-pial demyelination does correlate with the number of T- and B-cells in the leptomeninges[15,20,21,24,59,134], which may also migrate to the perivascular spaces via the penetrating meningeal blood vessels. This idea is supported by the finding that related clones of B lymphocytes are found in both the meningeal lymphoid structures and perivascular cellular infiltrates in the cortical and subcortical white matter[135]. B-cells are suggested to play both antibody dependent and independent roles in MS pathology and evidence exists for humoral immunity against neuronal and astroglial targets[136,137,138]. Locally produced autoantibodies can demyelinate explant cultures in the presence of complement[138], whilst complement can also be damaging in the absence of immunoglobulin[139]. Complement recognition molecules and products of activation are elevated in the MS CSF and complement is associated with synaptic, neuritic and myelin pathology in the MS GM[140,141]. Common variants in complement are associated with a more severe MS and complement C1q and C3b-d decorate cell soma, neurites and synapses for engulfment[142,143]. Limiting complement activation is effective in reducing synapse loss and preserving neurological function in EAE[144]. A reappraisal of the role of antibodies and

complement in the various grey matter compartments, and in cases displaying extensive inflammation of the meninges and perivascular space, is required.

Pathogenetic mechanisms linking meningeal inflammation and neurodegeneration

Elevated leptomeningeal inflammation is associated with a more extensive cortical demyelination, reduced cortical neuronal density, greater neuronal necroptosis and a shorter time to progression, substantial disability, and death (Figure 3). The extent of neuro-axonal damage correlates well with regional brain tissue atrophy and measures of clinical severity. However, as yet, the precise mechanisms by which inflammatory cells in the meninges and other connective tissue spaces contribute to neuro-axonal and synaptic damage remains to be fully resolved.

Studies of MS tissues at the earliest, acute, and inflammatory phase, revealed pyknotic neurons[24] and a decrease in neuron density in non-lesion (19.7%) and lesion (34.3%) cortical GM in cases harbouring elevated leptomeningeal infiltrates[49]. Neuron, neurite, and synapse loss are a hallmark of progressive MS, and it is estimated that there is the loss of 9.5 billion cortical neurons (39% reduction) in long-standing MS[145]. Cortical neuron densities and estimates of total neuron number correlated with cortical and white matter volume[145]. Neuron density is independent of the extent of grey and white matter demyelination and neuron loss can be substantial even in the absence of detectable WM lesions[146]. The select depletion of superficial populations of interneurons important in local circuit physiology is a component of neuron loss in cases with elevated leptomeningeal inflammation [5,147], but it is also clear that projection neurons are also reduced in number[5] and display reduced dendritic spine density and complexity[10,148]. Neurites and synapses are depleted in the MS cortex in vivo[10,20,149], which, when revealed by GABA-A receptor PET imaging with 11^{C}-flumazenil, correlates with cognitive performance[150]. Proinflammatory cytokines TNF and IFNγ can cause synapse loss and TNF, IFNγ and a range of other cytotoxic and or lymphoid-homing chemokines, are elevated in MS CSF as described[25]. Evidence for the effect of diffusible factors, emanating from the overlying leptomeninges and perivenular spaces is best illustrated by the presence of a gradient of relative neuron loss - greatest in the more superficial layers in comparison to deeper cortical laminae, that is only evident in cases characterised by leptomeningeal inflammation[5]. The relationship of this gradient to the CSF-filled space in cases with active inflammation, its presence distal to leptomeningeal aggregates and its distribution in the neocortex, thalamus, periventricular WM and spinal cord, suggests it is the action of diffusible factors that principally drives damage rather than the effect of cell-cell directed cytotoxicity[151,152,153,154,155].

Neurons of the MS cortex display evidence of reactive oxidative damage, and mitochondrial defects and insufficiency, which would contribute to an energy depleted state that is likely to compromise neurons attempting to survive a chronic and persistent environment of compartmentalised inflammation[156,157]. Products of CD20+ B effector cells isolated from MS CSF are directly toxic to cultured neurons and oligodendrocytes[158,159,160], whilst experimental and pathological evidence for a role for complement in neuronal injury in active progressive MS is supported by the finding of complement synthesis and deposition, alongside reduced complement regulator protein expression, in MS GM lesions[141,161,] and a key role for complement in synaptic degeneration[144]. Cytokines and complement, elevated in the CSF and parenchyma, may polarise microglia and astrocytes to acquire a reactive and damaging form[125,162]. Alongside those proinflammatory immune mediators already discussed, the MS CSF is enriched in bio-active lipids, including key products of cholesterol metabolism and ceramide, which can be directly neurotoxic[163], and may modulate glial activation or contribute to astrocyte-induced neural damage[164]. For example, bile acid metabolism is reduced in

MS and in this context tauroursodeoxycholic acid can inhibit damaging astrocyte and microglial responses[168]. Simvastatin, a cholesterol-reducing therapy with a complex and incompletely understood mode of action, is associated with a slowing of brain atrophy in SPMS and a preservation of regional cortical connectivity and cognitive resilience[166,167].

TNF is elevated in active MS lesions and in the CSF and meninges of patients and at post-mortem, where it associates with GM pathology. Bulk microarray gene expression analysis of macrodissected motor cortex from cases with and without leptomeningeal inflammation revealed gene expression changes suggestive of a dysregulation of TNF mediated cell death signalling[168], which could be responsible for driving neuronal damage. Pathways linked to the action of soluble TNF, rather than membrane-bound TNF, that drive death-signalling via interaction with TNF receptor 1 and involving the RIPK1/3 and mixed lineage kinase domain-like (MLKL) kinase cascade, were upregulated in MS GM characterised by leptomeningeal inflammation. MLKL phosphorylation, oligomer formation and membrane insertion is required for necrosome formation – an essential step in necroptotic cell death. Necroptosis, rather than apoptosis, which is rarely observed in MS neurons, is suggested to be the overriding pathway by which neurons degenerate. This interpretation is based on the significant expression of necroptotic markers, and the parallel downregulation of cleaved caspase-8 required for apoptosis[169,170]. The sustained production of TNF in the subarachnoid space may stimulate degeneration of cortical neurons in the post-mortem MS brain, particularly those of the outer layers, with the biochemical signature of necroptosis[170,97], and this has been reproduced in a rat model of MS cortical pathology involving chronic expression of TNF in the meninges95. Lymphotoxin-alpha (LTα) is amongst the inflammatory cytokines that associates with a more severe clinical and pathological outcome[25,26]. LTα, which binds TNFR1 and 2, is important in lymphoid neogenesis in other organ systems and is associated with a worse MS outcome (figure 2). Chronic induced LTα synthesis in the SAS in rats is sufficient to cause meningeal inflammation with lymphoid tissue formation, microglial activation, and neuronal loss by necroptosis[96]. These investigations bridge descriptive clinical and pathological studies to highlight how significant a TNFR1-driven neurodegenerative pathology might be to MS disease pathogenesis (Fig 2).

Association of meningeal inflammation with worsening clinical course

Even if limitations in intact meningeal collection/preservation from post-mortem MS brain tissue has not always allowed the observation of cortical lesions in relation to the presence of meningeal inflammation[44, 110, 173], a strong correlation between meningeal inflammation and the degree of neuro-axonal injury and/or dysfunction and MS clinical outcome has been widely demonstrated[5,11,22,171]. However, the extent to which sub-pial demyelination gives rise to clinical symptoms is unclear. It is likely that the deeper through the cortical layers the demyelination penetrates then the greater the possibility of symptoms. Deficits in layer I connectivity due to demyelination may not be noticeable, but slowing of AP conduction in association fibres from layer II-IV neurones would be expected to have an effect depending on the extent and location of the lesions. Neuronal loss is much more likely to give rise to permanent symptoms depending on the extent and location of the loss. But how many neurons need to be lost before clinical symptoms arise? It is likely that clinical symptoms will only occur when a significant proportion of neurons have been lost and plasticity exhausted, as is seen in Parkinson's disease. This proportion may vary considerably depending on the cortical areas affected. However, it is inevitable that a slow build-up of cortical neuron loss as a result of meningeal inflammation would eventually lead to irreversible disability, which may be a combination of motor, sensory and cognitive dysfunction. Severe cognitive impairment can be the primary disabling

manifestation of MS, without any other significant neurological impairment[172] and is presumably due to irreversible cortical neuron or axon loss. Psychiatric symptoms (65%) and other diverse cortical signs and symptoms may also have the same pathological substrate (e.g. seizure, aphasia, apraxia) (39%). Therefore, in addition to acute WM lesion and expansion of chronic active lesions, increasing meningeal inflammation may represent one of the key factors contributing to increased disability, most likely via the stimulation of cortical GM pathology[3,21,155]. However, linking the anatomical location of the pathology to specific symptoms and disabilities is extremely difficult. Additional factors, including environmental and genetic individual background, come possible into play[18].

Measures of meningeal inflammation

Clinical features of MS for monitoring meningeal inflammation

Significant meningeal inflammation associated with subpial cortical demyelination and neuronal degeneration can occur early during the initial stages and early years of MS[24,49]. Therefore, it is important to identify clinical correlates of these pathological processes in order to follow their development. However, there are currently no objective clinical features that can be used as correlates of MS meningeal inflammation. Accumulating studies suggest that persistent headache may represent a potential feature of MS during its initial stages and the prevalence of headaches in MS patients is significantly higher than in controls. The prevalence of migraine in MS patients varies between 43.3% up to 71.8%, compared to approx. 10% in the normal population[174]. However, the idea that acute meningeal inflammation gives rise to headaches in MS needs to be substantiated before it can be used as a clinical marker.

The strong relationship between the presence of oligoclonal bands (OCB) in the CSF of MS patients, which for decades has been recognized as an immunopathological key feature and diagnostic marker of MS, and cortical demyelination in MS[175], support the hypothesis of potential correlation between meningeal inflammation, particularly enriched in B cells, and OCB. This relationship would confirm the idea that meningeal immune cell infiltrates may represent niches for intrathecal B cell expansion and perpetuation of plasma cell activity and, therefore, production of immunoglobulins, together with inflammatory factors.

CSF biomarkers of meningeal inflammation

Given the new insights into the influence of meningeal inflammation on MS pathology, analysis of CSF is likely to provide insights into the pathogenetic mechanisms underlying this compartmentalised immune response. In addition, it should allow the identification of suitable biomarkers and useful tools to monitoring this feature throughout the course of MS. Cell and cytokine profiling of MS cerebrospinal fluid (CSF) has demonstrated increases in the levels of B-cell attractant chemokines, such as CXCL13, together with increases in B cell populations under conditions of an intact BBB [176], suggesting a possible intrathecal environment that can further influence recruitment and differentiation of different immune cell populations. In contrast, under conditions of a disrupted BBB, NK cells significantly increased and correlated with a more complex CSF protein pattern[176], suggesting a complex interaction between the intrathecal cascade of cytokine expression and different phases of MS neuroinflammation. Increased CXCL13 CSF levels in MS patients have been found to be also associated with the frequency of CXCR5+CD4+ T cells, also known as professional T follicular helper cells, that could have an important role in initiating and maintaining B cell immunity in the intrathecal space[177]. More recently, increased levels of follicular helper T (Tfh) cells, crucial to support B-cell differentiation in secondary lymphoid organs, were have been identified in the CSF of MS patients at time of diagnosis, but not in the serum, of MS patients at the time of diagnosis[178],

suggesting that these cells might have a key role in promoting TLS development and intrathecal B cell activity.

Recent studies have demonstrated good correlations between CSF and meningeal inflammatory profiles, which have been further validated using novel experimental rat models[92,25,95,96]. A specific CSF protein pattern, including high levels of CXCL13, IFNγ, TNF, CXCL12, IL6, IL10 and LIGHT, has been demonstrated to be able to predict 89% of the variance in cortical lesion volume/number and increased disease activity, both at the time of diagnosis in drug naïve MS patients, and at time of death in post-mortem MS cases[25,26]. A 4 year follow up on the same patient cohort confirmed this association, proving a good rationale for using a combination of pro-inflammatory CSF markers to confirm the presence of increased meningeal inflammation and worsening disease prognosis. All the above studies suggest a direct relationship between the extent of meningeal inflammation and the extent of grey matter damage and disease outcome. Since meningeal inflammation imaging is still not MS-specific and has not yet been validated as a reliable tool to detect MS-specific meningeal lymphoid-like structures[179], a specific CSF molecular profiling[25,26] represents a surrogate marker of meningeal inflammation and might help to early identify in-vivo the presence of meningeal inflammation. This in turn will also help to detect diffuse subpial cortical demyelination associated with meningeal inflammation and might be effective in early distinguishing of MS subtypes at high risk of severe cortical damage and rapid disease progression.

MRI correlates of meningeal inflammation

MRI cortical lesion detection

Since its first observations, meningeal inflammation has been topographically associated with subpial cortical demyelination and cortical atrophy at post-mortem[20-24] and in ex-vivo biopsy tissue studies[24]. A spatial relationship between the ectopic lymphoid-like tissues was found adjacent to subpial type-III lesions, suggesting a possible relationship between their formation and cortical damage and that soluble factors diffusing from these structures have a pathogenic role. Therefore, several MRI studies have focused on the identification, in vivo, of cortical lesions as a possible marker of meningeal inflammation. The use of new non-conventional MRI allowed confirmation that cortical lesions were not exclusive to the progressive stage, but appeared early during the disease process[180,181], sometimes already at clinical onset. In line with the seminal neuropathology studies identifying meningeal B cell rich lymphoid-like infiltrates, several MRI studies identified cortical lesions and GM atrophy among the major predictors of a severe disease clinical course[182,183,184]. In those studies, the rate at which cortical lesions accumulated was associated with the overall disease severity and could most likely be used to predict early disease progression and irreversible disability accumulation[183]. Thus, the presence of cortical lesions has been suggested to confirm the diagnosis of MS[185] and to identify patients at high risk of physical and cognitive disability progression. However, imaging of cortical lesions has always been considered challenging because they are usually small and have slight differences in their relaxation times compared to the normal-appearing GM[183]. This characteristic and the partial volume effects from the adjacent cerebrospinal fluid result in poor contrast resolution between them and the surrounding normal GM. Although during recent years, the introduction of double inversion recovery (DIR) and phase-sensitive inversion recovery (PSIR) sequences has improved the detection of CLs in MS patients, most of them (especially type 3 subpial lesions) still escape identification even with high field MRI[186,187].Diffuse GM atrophy, which occurs early in MS and increases during disease progression, reflecting disability accumulation[184,188,189], may represent one of the best surrogate markers of widespread inflammation within the leptomeninges.

MRI evidence of a surface-in gradient of pathological changes

Concomitant neuropathological and imaging findings support the existence of an MS-specific "surface-in" spatial distribution of abnormalities in both WM and GM lesions and normal-appearing brain regions. Both outer periventricular and subpial cortical layers show abnormalities that decrease with distance from the CSF, as assessed either by magnetisation transfer ratio (MTR) or diffusion tensor imaging (DTI) imaging methodologies[151,152,190,191]. This has also been observed in the spinal cord[192] and in pediatric MS[153,154]. A periventricular gradient of innate immune system activation, detected by MRI and (18F-DPA714G) dynamic PET has been demonstrated in the periventricular lesions and normal-appearing WM of MS patients with disability worsening[193]. Neuropathological studies of subpial cortical and periventricular thalamic pathology revealed that the highest neuro-axonal loss and microglial activation was present in the external cortical layers close to the CSF boundaries, in MS cases compared to healthy donors[5,194]. All these superficial, microstructural pathological alterations have been found to be enhanced in association with the presence of elevated inflammation and lymphoid-like structures in the meninges[5,155].

Similar to findings in the cerebral cortical GM, a thalamic gradient of neuronal loss and microglial activation was associated with a specific CSF composition, including neurodegeneration markers (NfL and parvalbumin), glial activation markers (chitinase-3-L1, sCD163), proinflammatory mediators (sTNFR1, TNF, fibrinogen, IFNγ) and several lymphoid chemokines (CCL19, CCL21, CCL22, CXCL10, CXCL13)[155] . In addition, deposition of fibrinogen on neuritic/glial process was found to be greater in MS in cortical layers 1 and 2 in post-mortem MS compared to control cases[195].

All the above data support the hypothesis that inflammatory events occurring in meninges, and possibly in the choroid plexus, have a key role in regulating the composition of the CSF and intrathecal inflammatory environment. Inflammatory and/or cytotoxic mediators locally expressed by meningeal inflammatory infiltrates can diffuse throughout the pial and ependymal surfaces and mediate/enhance the "surface-in" gradient of grey matter damage observed only in MS and not in other neurological conditions[5,196].

Leptomeningeal enhancement as an MRI marker of meningeal inflammation

More recently, leptomeningeal enhancement (LME), which can be detected by gadolinium-enhanced high-resolution fluid-attenuated inversion recovery (FLAIR) MRI sequence, has been proposed as a new imaging marker of meningeal inflammation. A delayed post-contrast acquisition, at least 10 minutes after the intravenous administration of gadolinium, characterizes this FLAIR that was significantly better than conventional T1-weighted imaging, providing as much as 10-fold increased sensitivity in the detection of low concentrations of contrast in the subarachnoid space[197].

In the first study, LME was found in only 1 of 112 patients (0.9%), suggesting that LME was generally uncommon during the relapsing-remitting early stages of MS (Eisele et al, 2015). However, using a more advanced high-resolution 3D T2 FLAIR MRI with a voxel size of 1.0 × 1.0 × 1.0 mm. Absinta et al.[199] found that LME was significantly more common than initially reported, as the authors observed LME in 74 of 299 patients with MS (24.7%) compared with only 1 of 37 (2.7%) age-matched controls without MS. They also showed that LME was associated with patient age, disease severity, and clinical type of MS, being much more frequent (33%) in patients with progressive MS forms compared with those with RR disease (19%).

Further independent studies[200,201] and the application of ultra-high field MRI then confirmed the high frequency of LME in MS patients[202], also describing two distinct LME patterns: "nodular" and

"spread/fill." Nodular foci appeared as small, discrete nodules of contrast either at the pial surface or in the subarachnoid space; they were usually small and spherical-shaped. Spread/fill foci appeared as larger, nebulous areas of contrast in the subarachnoid space, observed in 76% of subjects. MS subjects with spread/fill foci were older than those without, whereas those with nodular foci present were slightly younger than those without. Spread/fill foci, on the other hand, were not seen in any healthy volunteers, and their presence was associated with reduced cortical volumes, all supporting the notion that this pattern is pathologic and associated with cortical pathology. However, leptomeningeal compartment contrast enhancement appears not to be specific to MS. In recent work, Absinta and colleagues[203]demonstrated that LME was 4-fold more frequent in inflammatory and immune-mediated neurologic conditions (35%) than in non-inflammatory neurologic conditions (8%) and healthy volunteers (8%). Other studies found LME in other inflammatory non-MS conditions[204,205,206] such as Susac syndrome, Neurosarcoidosis, and Rheumatoid meningitis (a rare and diagnostically challenging manifestation of rheumatoid arthritis) and also in the NMO[207]. In the latter case, the antibody to AQP4 binds to the surface of microvessels, pia, and Virchow–Robin sheaths and damages the astrocytes. Thus, leptomeningeal enhancement is probably a result of functional impairment of AQP4 water channels in the pial and subpial surfaces. The low frequency of LME in controls and individuals without underlying inflammatory neurologic disease provides additional support to the recent notion that CSF-restricted enhancement on postcontrast T2-FLAIR images, when present, is an expression of the breakdown of the blood–meningeal barrier, related directly to ongoing inflammation or post-inflammatory scarring, as might occur in traumatic brain injury[203,208]. This interpretation is in line with the role of the leptomeninges as a relay and modulatory gate for peripheral immune cells in health and in a variety of immunopathologic processes that lead to focal blood–meningeal barrier impairment. It is also very unlikely that LME is providing a signal from the large lymphoid-like immune cell aggregates that accumulate in the MS meninges[20], which would not be expected to exhibit acute immune cell influx that would give rise to gadolinium enhancement. Thus, it may have limited use as a marker of the extensive meningeal inflammatory infiltrates seen in pathology studies.

Therapeutic implications of meningeal inflammation in MS

Targeting LTα function

Given the key role of LTα in TLO development, targeting the LTα1β2-LTβR signalling pathway was proposed to modulate TLO formation. Pateclizumab (a monoclonal antibody against LTα) and baminercept (LTβR-IgG1, an inhibitor of both the LTα1β2 and LIGHT pathways) have been investigated in Phase I and II trials for the treatment of autoimmune diseases[209,210]. In addition, ongoing studies on experimental animal models are examining the potential therapeutic strategies by blocking molecules involved TLO formation, such as anti-IL21, anti-IL-17 and anti-ICOS, inhibiting the accumulation of immune cells in models of rheumatoid arthritis[211].

Blocking CXCL13

Aberrant expression of the major B cell chemoattractant molecule CXCL13 within lymphoid-like structures suggests that antibody-mediated disruption/block of the CXCL13 signalling pathway could inhibit the formation of meningeal lymphoid immune cell aggregates in the target organs and inhibit chronic inflammation[212]. Blockade of the chemokine CXCL13 was shown in several mouse models to reduce glandular inflammation in Sjogren's syndrome model[213] and decrease the severity of collagen-induced arthritis and GC formation in synovial tissues[214]. The use of a novel human anti-

human CXCL13 antibody, MAb 5261, has demonstrated efficacy in two well-characterized mouse models of autoimmunity: CIA (both prophylactic and therapeutic models) and passively and actively induced models of relapsing-remitting EAE[212]. However, anti-CXCL13 antibodies have yet to be tested therapeutically in MS.

Anti-B cell therapies

Several clinical trials directly targeting B cells, such as rituximab, ocrelizumab, ofatumumab, and ublituximab, have recently demonstrated their efficacy in RRMS and, more moderately in PPMS, inducing a significant reduction of disease activity and disability progression[215,216,217,218,221]. Treatment of MS patients with anti-CD20 has demonstrated early and persistent decreases in CSF B cells and CXCL13 levels after 52 weeks[219]. In addition, it was suggested that meningeal lymphoid-like structures do not contain only CD20+ cells but also plasmablasts and plasmacells that would not be affected by anti-CD20 therapies[220]. Administration of intrathecal rituximab in progressive multiple sclerosis patients demonstrated transient reductions in CSF B cells and CXCL12, CXCL-13 and BAFF levels, but without evidence of reduction of leptomeningeal contrast enhancement[222,223] and without any biomarker to demonstrate a reduction in TLO-like structures.

Considering the size of monoclonal antibodies, it remains still unclear whether these effects are due to a real intrathecal effect or, indirectly, to the reduced B cell peripheral inflammation and therefore diminished recruitment within the CNS.

Considering the size of monoclonal antibodies, it remains still unclear whether these effects are due to a real intrathecal effect or, indirectly, to the reduced B cell peripheral inflammation and therefore diminished recruitment within the CNS. It is possible that monoclonal antibodies could reach the CNS by alternative routes not characterized by the presence of the classic BBB structure, such as the Blood–Meningeal Barrier or the Blood CSF Barrier. However, it remains mandatory to develop novel tehnical approaches allowing any kind of antibody therapy to achieve their effects within the CNS, in particular when the BBB is undamaged.

BTK Inhibitors

Bruton's tyrosine kinase (BTK) is a cytoplasmic enzyme involved in the signalling and maturation of B cells and myeloid cells[224]. Preliminary studies of the effects of BTK inhibition on meningeal inflammation in the SJL model of EAE demonstrated reduced meningeal contrast enhancement on ultra-high field MRI and reduced the number of B cells within areas of meningeal inflammation[224]. At the same time, reduction of new enhancing lesions in phase 2 placebo-controlled trial in RRMS patients of varying doses of the oral BTK inhibitor was also demonstrated[226].

Novel therapeutic strategies

A recent study suggested that the second-generation sphingosine phosphate 1 receptor modulator with high affinity to S1PR1 and S1PR5, Siponimod, can reduce the development of spinal cord meningeal inflammation in mice with TCR and BCR specific for myelin proteins[227]. In line with evidence of EBV-infected B cells in the meninges and perivascular inflammatory infiltrates of the CNS, clinical improvement was observed in most of the MS patients (6 out of 10) treated with CD8+ T cells expanded in vitro and targeted against EBV antigens[228].

Conclusions/perspectives

Aberrant chronic meningeal inflammation plays a key role in MS immunopathology as one of the major drivers of subpial cortical pathology and associated severe clinical progression and accumulating disability. This highlights three important needs: 1) further understanding of the cell and molecular pathogenetic mechanisms involved in meningeal inflammation in order to identify specific new targets for therapeutic translation; 2) improved neuroimaging methods and fluid-based biomarkers for early detection and continuous monitoring in vivo; and 3) new methods for intrathecal delivery of therapeutics. For these reasons it is particularly important to identify the most appropriate animal models mimicking the development of meningeal inflammation for translational studies, as well as the most useful cell and in silico models of immune cell recruitment and TLO formation that reproduce the inflammatory environment of the MS subarachnoid space. Identifying rational new drug targets that inhibit the pathological processes that lead to the development and maintenance of compartmentalised inflammation of the CSF space should provide much needed advances in our ability to treat the progressive phase of MS.

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Author contributions

All the authors wrote the article. R.M., O.H. and R.R. researched data and images for the article. R.M., O.H., M.C. and R.R. made substantial contributions to discussion of the content and reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Figure legends

Figure 1. Cortical demyelination and leptomeningeal inflammation in MS: an historical summary. (A- C) Areas of leukocortical and subpial demyelination described by James W. Dawson (1921) are now readily viewable on immunostained whole brain coronal sections (D; Griffiths et al., 2020), where infiltrates of CD3+ and CD20+ lymphocytes are seen within the confines of the sulcal leptomeninges (E, F). Leptomeningeal inflammation is described at, or near, sites of subpial demyelination (G, H). (I, J) significant leptomeningeal and connective tissue infiltrates in PMS (from Guseo and Jellinger. 1975) follicle-like structure in the MS leptomeninges comprising an aggregate of CD20+ B-cells (K) and a reticular network of follicular dendritic cells expressing CD35 (L) and proliferating B-cells (M; K- M from Serafini et al., 2004). All images reproduced with permission.

Figure 2. Meningeal lymphoid-like structures in MS and animal models. A: Neuropathological immunostaining of myelin oligodendrocyte glycoprotein (MOG) identifies subpial type III cortical lesions adjacent to inflamed meninges containing a lymphoid-like structure with high number of CD20+ B cells and CD3+ T cells. Interestingly, a portion (limited by dash dot line) of such immune aggregate is enriched in B cells compared to T cells. Leptomeningeal lymphoid-like structures may

contain numerous proliferating Ki67+ B cells and scattered CD27+ memory B cells, in presence of lymphoid chemokine CXCL13 and cytokine LTα, that play a key role in the recruitment and organization of immune cell in lymphoid neogenesis (A). B: Neuropathological features of lymphoid-like structures in animal models. DAPI nuclear staining shows the accumulation of cells down the entire length of the sagittal sulcus in both IFA and MOG immunised rats (at 28 and 90dpi) after injection lymphotoxin-alpha lentiviral vector into the subarachnoid space. Immunostaining shows the expression of mucosal addressin cell adhesion molecule (MAdCAM-1) by the majority of the larger lymphatic-like channels and some smaller HEV-like vessels, together with the major B-cell chemoattractant chemokine CXCL13. High number of CD4+ and CD8+ T-cells, CD79a+ B-cells and IBA1+ myeloid cells were identified within the dense infiltrates into the SAS. Reduced numbers of HuC/D+ neurons were observed in cortical parenkyma of rats injected with lentiviral vectors (LVs) expressing enhanced LVLTα animals compared to LVGFP. HuC/D+ or NeuN+ neurons were dying via necroptosis, as shown by the expression of phosphorylated MLKL, which is the final protein involved in the necroptosis pathway (B; from James-Bates et al., Brain 2022 and Picon et al., Acta Neuropathol 2021). C: Schematic depiction of the physiological and pathological events associated with MS leptomeningeal infiltration. While in physiological conditions scattered immune cells circulate within the subarachnoid space in the CSF and in the leptomeninges (blue circle), in presence of chronic MS-specific intracerebral inflammation (red circle) increased expression of lymphoid chemokines (CXCL12, CXCL13, CCL19, CCL21) and proinflammatory molecules (LVLTα, BAFF, IFNγ, TNF, IL1β) by resident stromal cells, border and infiltrating macrophages, follicular dendritic cells and possibly by brain parenchymal cells, mediate the recruitment, proliferation and survival of an abnormal number of T-cells, B-cells, macrophages, plasma cells and dendritic cells in the leptomeninges. These meningeal infiltrates, spead along the cerebral sulci and/or organized in ectopic tertiary lymphoid-like nodular structures, contributing to persistent intracerebral antigen presentation, antibody production and expression/release of inflammatory and cytotoxic mediators may be involved in tissue damage of the adjacent cortex either directly or indirectly, by stimulating glia activation/changes, for example by inducing the MLK-mediated necroptosis pathway. All images reproduced with permission.

Figure 3. Leptomeningeal inflammation and the pathological and clinical burden of disease. Semi-quantitative assessment of leptomeningeal inflammation (0 – 3; 3 equals significant infiltrates of cells and one or more lymphoid-like structures) revealed 50.2% of assessed cases of PMS in the UK MS Tissue bank presented with moderate (2) or substantial (3) leptomeningeal inflammation (A). Those displaying moderate or substantial infiltrates transitioned to the progressive phase at a younger age (B), displayed disproportionally greater cortical grey matter demyelination (C), more active (active or chronic active) inflammatory demyelinating lesions (D) and were present to a similar extent in males and females (E). The presence of moderate to substantial cellular infiltrates was characteristic of cases with a younger age of MS onset, age to progression, age at substantial disability (when a wheelchair was required), a shorter disease course and a younger age of death (F- J). Data based on the review of 217 cases of progressive MS. Kaplan-Meier analysis, Kruskal-Wallis and Dunn's post-test (3-group comparisons) or Mann-Whitney U test analysis.

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Figure 1

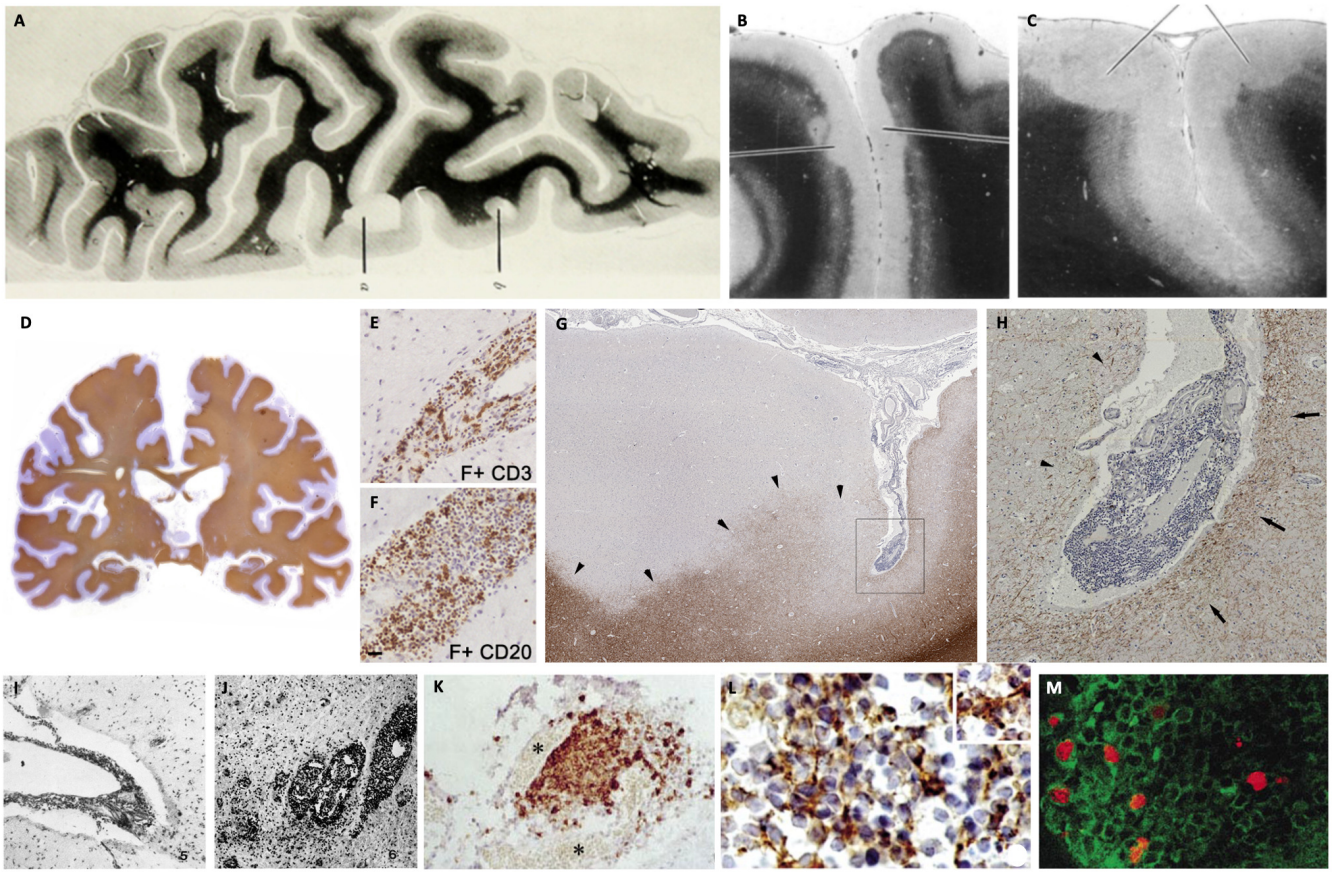

Figure 2

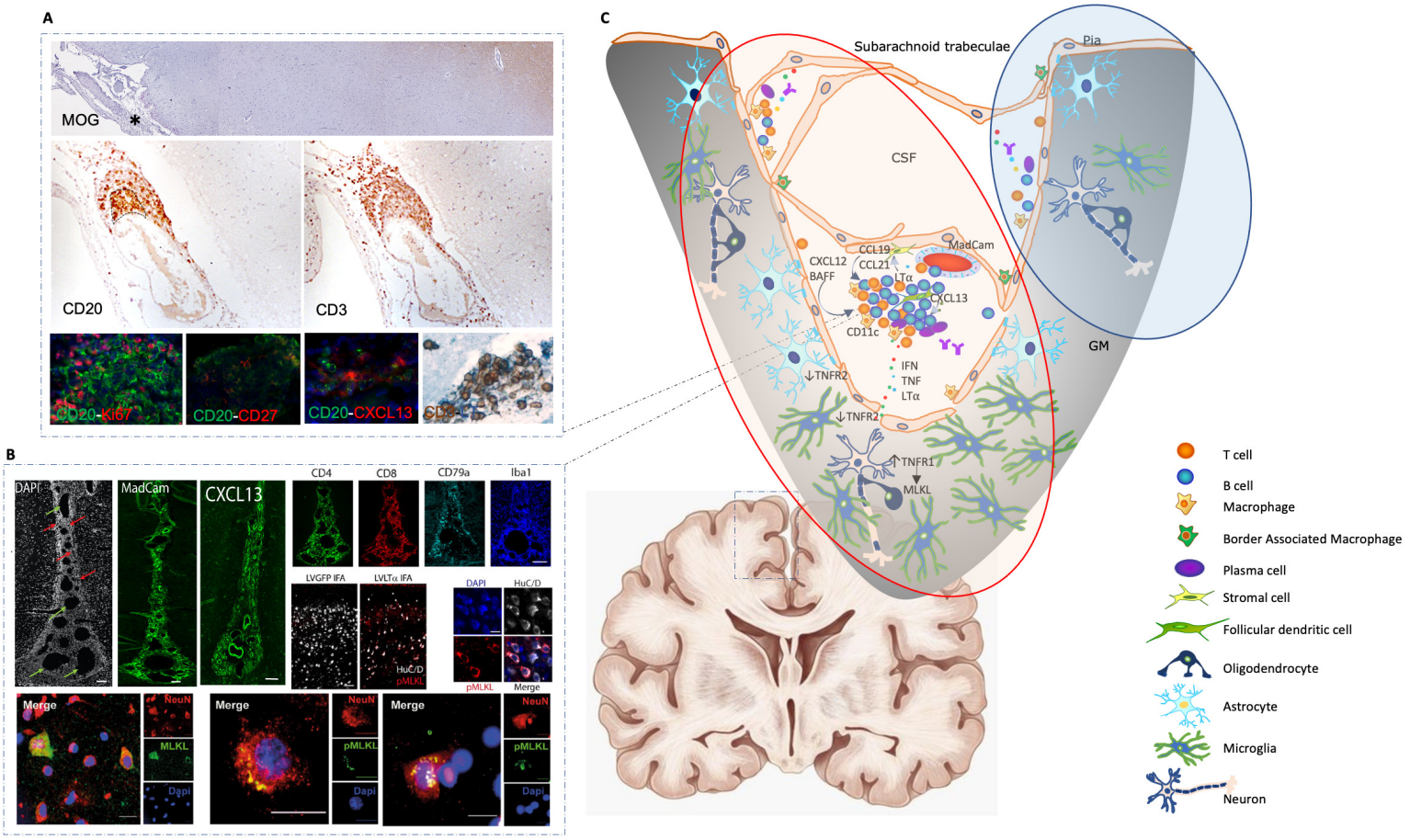
A
MOG
*
CD20
CD3
CD20-Ki67
CD20-CD27
CD20-CXCL13
CD3-CD20
B
DAPI
MadCam
CXCL13
CD4
CD8
CD79a
Iba1
LVGFP IFA
LVLTα IFA
DAPI
HuC/D
HuC/D
pMLKL
pMLKL
Merge
Merge
NeuN
MLKL
Dapi
Merge
NeuN
pMLKL
Dapi
Merge
NeuN
pMLKL
Dapi
C
Subarachnoid trabeculae
Pia
CSF
CCL19
CCL21
MadCam
CXCL12
BAFF
LTα
CXCL13
CD11c
GM
IFN
TNF
LTα
↓TNFR2
↓TNFR2
↑TNFR1
MLKL
T cell
B cell
Macrophage
Border Associated Macrophage
Plasma cell
Stromal cell
Follicular dendritic cell
Oligodendrocyte
Astrocyte
Microglia
Neuron

Figure 3

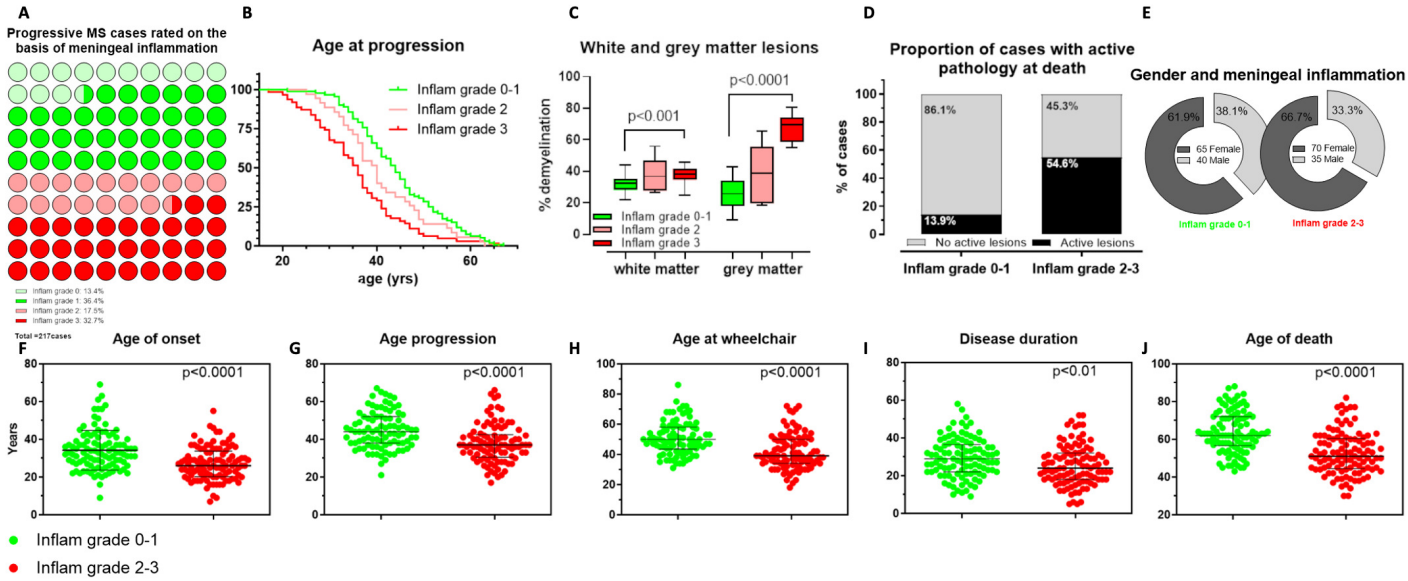
A
Progressive MS cases rated on the basis of meningeal inflammation
Inflam grade 0: 13.4%
Inflam grade 1: 36.4%
Inflam grade 2: 17.5%
Inflam grade 3: 32.7%
Total =217cases
B
Age at progression
Inflam grade 0-1
Inflam grade 2
Inflam grade 3
age (yrs)
C
White and grey matter lesions
p<0.001
p<0.0001
% demyelination
Inflam grade 0-1
Inflam grade 2
Inflam grade 3
white matter
grey matter
D
Proportion of cases with active pathology at death
% of cases
86.1%
13.9%
45.3%
54.6%
No active lesions
Active lesions
Inflam grade 0-1
Inflam grade 2-3
E
Gender and meningeal inflammation
61.9%
38.1%
65 Female
40 Male
66.7%
33.3%
70 Female
35 Male
Inflam grade 0-1
Inflam grade 2-3
F
Age of onset
p<0.0001
Years
G
Age progression
p<0.0001
H
Age at wheelchair
p<0.0001
I
Disease duration
p<0.01
J
Age of death
p<0.0001
Inflam grade 0-1
Inflam grade 2-3

Table 1
Summary of the key studies on human meningeal inflammation.

Study	Principal finding
Serafini et al.,2004[19]	Aggregates enriched in proliferating Ki67+ B cells, plasma cells and CD35+CXCL13+ follicular dendtritic cells resembling ectopic TLOs in the meninges of MS cases.
Magliozzi et al., 2007[20]	TLOs identified in the inflamed meninges of 41% of progressive post-mortem MS cases in association with extensive subpial cortical lesions.
Serafini et al., 2007[61]	Identification of EBER transcripts and EBV antigens expressed by B cells in meningeal TLOs of progressive MS brains but not in non-neurological controls.
Kooi et al., 2009[44]	Identification of mild meningeal inflammation in 28 MS cases but did not find any TLOs with associated subpial cortical lesions.
Frisher et al., 2009[63]	Identification of meningeal immune cell infiltrates in 22% of MS cases in association with active demyelination. Plasma cell numbers highest in the meninges.
Willis et al., 2009[106]	EBV protein and gene expression only very rarely found in tissues from 24 MS brains. No meningeal TLO-like structures were found.
Magliozzi et al., 2010[5]	A surface-in gradient of cortical neuronal loss and microglia activation in MS cases with meningeal TLO-like tissues but not in those without.
Torkildsen et al., 2010[110]	Upregulation of immunoglobulin-related genes, but absence of EBV protein and gene expression in meninges and cortical GM in MS brains.
Peferoen et al., 2010[107]	Prominent meningeal B cell infltrates observed in 16 MS cases, but without evidence of TLO formation ofr the presence of EBV encoded RNA and EBV lytic and latent antigens proteins.
Lucchinetti et al., 2011[24]	Substantial meningeal inflammation, enriched in CD3+ T cells and CD20+ B cells, in 30% of MS cortical biopsy samples from acute MS patients. Infiltrates were closely associated with cortical demyelination.
Howell et al., 2011[21]	TLO-like lymphoid tissues identified meninges of 40 % of 129 MS brains always in association with extensive subpial cortical lesions and more rapid disease progression.
Lovato et al., 2011[62]	Clonally expanded of B cells from the same clones found in 28% of meningeal immune cell infiltrates and 24% of parenchymal infiltrates in MS brains.
Choi et al., 2012[50]	Identification of elevated meningeal inflammation in 31% of PPMS brains but without evidence of TLO organization.
Magliozzi et al., 2013[133]	Expression of EBV EBER transcripts and BZLF1 antigen in meninges and small perivascular cortical infiltrates in 26 MS brains with meningeal TLOs but not in those without.
Howell et al., 2015[15]	Mild or substantial meningeal inflammation identified in the meninges in the cerebellum of 27 MS brains in association with

	subpial cortical demyelination but without evidence of TLO organization.
Haider et al., 2016[22]	High incidence of large meningeal inflammatory infiltrates in a substantial proportion of 51 MS brains in association with underlying subpial cortical lesions.
Bevan et al., 2018[49]	Evidence of B-cell rich, CXCL13-expressing, meningeal TLO-like tissues in 33% of acute MS brains with short disease duration.
Magliozzi et al., 2018[25]	Increased expression of proinflammatory molecules related to B-cell activity and lymphoid-neogenesis was detected in the meninges and CSF of 10 MS brains with lympoid-like infiltrates compared to those without and control brains.
Hassani et al., 2018[105]	Identification of EBV antigens in infiltrates in the meninges of 47% of MS brains with mild meningeal inflammation.
Veroni et al., 2018[102]	Laser capture and transcriptomics of meningeal lymphoid-like tissues revealed EBV related gene expression in most infiltrates and genes related to B-cell differentiation and type 1 interferon activation pathways.
Bell et al., 2019[59]	Evidence of meningeal inflammation with TLO-like organisation in 22 SPMS and 11 PPMS brains but with lack of FoxP3+ regulatory T cells.
Magliozzi et al., 2019[168]	Transcriptomics analysis of subial cortical lesions in MS brains with TLO-like tissues and those without and controls revealed an enhanced TNF/TNFR1 signalling that was shifted towards necroptosis in brains with meningeal TLOs.
Griffiths et al., 2020[109]	Extensive sampling of MS brains with above-median extent of cortical demyelination displayed a substantially increased median rating of leptomeningeal inflammation, including TLO-like infiltrates.
Reali et al., 2020[16]	TLO-like lymphoid tissues were present in the spinal cord meninges of MS cases characterised by TLO-like infiltrates in the cortical meninges and the degree of B-cell infiltration was corelated with extensive spinal cord axonal loss.
Junker et al., 2020[18]	Subpial cortical lesions were found specific to MS brains and not present in other non-MS demyelinating and non-demyelinating inflammatory CNS disorders, but were not always in association with evident meningeal inflammation.
Van Olst et al., 2021[6]	Elevated meningeal inflammation in MS brains was associated with a shift of cortical microglia to a pro-inflammatory phenotype associated with neurodegeneration.
Picon et al., 2021[170]	Prominent meningeal inflammation was found associated with a 30-fold increase in activation of necroptosis in neurons in cortical layers I-III in MS brains compared to non-neurological controls.
Fransen et al., 2021[221]	Significant B-cell and plasma-cell infiltrates present in the medulla, subcortical lesions and biopsied lesions in MS brains. Absence of B-cells and lower numbers of meningeal B-cells correlated with a longer milder disease duration.
Ahmed et al., 2022[17]	Meningeal accumulations of T cells and B cells, but not myeloid cells, in 27 examined post-mortem MS cases was found associated with to large subpial cortical lesions, greater proportion of active

	and mixed active/inactive WM lesions and lower proportion of inactive and remyelinated WM lesions.
Magliozzi et al., 2022[155]	Increased ependymal-in gradient of neuroaxonal loss and microglial activation in chronic active thalamic lesion of MS cases with meningeal TLOs compared to those without and non-neurological controls.