2 PATHOLOGICAL DIFFERENCES BETWEEN WHITE MATTER AND GREY MATTER MULTIPLE SCLEROSIS LESIONS

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ABSTRACT

Multiple Sclerosis (MS) is a debilitating disease and is characterized by demyelination of the central nervous system (CNS) resulting in the wide-spread formation of white matter lesions (WML) and grey matter lesions (GML). WML are pathologically characterized by the presence of immune cells that infiltrated into the CNS, whereas in GML these are hardly present. This striking pathological difference between WML and GML questions the underlying mechanism. It is known that infiltrating leukocytes contribute to the generation of WML, however, since GML show a paucity of infiltrating immune cells, their importance in GML formation remains to be determined. Here, we review pathological characteristics of WML and GML, and put forward some possible explanations for the observed pathological differences. In our view, cellular and molecular characteristics in WM and GM, and local differences residing within in WML and GML, in particular by glial cell populations and their molecules expressed, determine the pathway to demyelination. Further understanding of GML pathogenesis, considered to contribute to chronic MS, may have direct impact on the development of novel therapeutic targets to counteract this progressive neurological disorder.

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INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating disease of the central nervous system (CNS) and is the most common cause of nontraumatic neurological disability in young adults.[22] It is pathologically characterized by disruption of the blood-brain barrier, influx of leukocytes into the CNS leading to a local inflammatory environment, and demyelination resulting in loss of conductance velocity within the axons.[207] Ultimately, this results in loss of functionally relevant axonal tracks accounting for the neurological symptoms.[203] These symptoms of MS can vary during the course of the disease from sensory symptoms, e.g. disturbed vision and sensation,[7,8,10,11] mobility-related symptoms, e.g. spasticity, ataxia or tremor,[12–16] to fatigue[337–339] and cognitive dysfunction.[20,188] The disease course is variable among affected subjects, but the majority of MS patients have a biphasic disease course, starting with relapsing-remitting MS (RR-MS), during which patients experience alternating episodes of clinical symptoms and complete or incomplete recovery. Subsequently, the disease may transform into a secondary progressive disease course (SP-MS) which is characterized by gradual worsening of the neurological symptoms.[22]

Traditionally, MS was regarded as a demyelinating autoimmune disorder resulting from the lack of discrimination between self-antigens, i.e. myelin, and foreign antigens and would subsequently lead to demyelination and death of oligodendrocytes mediated by infiltrating leukocytes.[340,341] Recent observations, however, have challenged this idea because demyelinated WM areas are not always associated with infiltrating leukocytes in early MS[342,343] and in a subset of MS patients with oligodendrogliopathy.[289,344] Moreover, no specific auto-antigen has been found yet and thusfar only modest success has been booked in delaying disability progression with established β-interferon therapy that suppresses the adaptive and innate auto-immune response.[35] These observations have led to the hypothesis that a mechanism other than, or in addition to, autoimmunity is involved in the pathogenesis of MS. It has been proposed that inflammation in MS is a secondary phenomenon that occurs as a response to primary oligodendrocyte dysfunction.[45,345]. In favour of this hypothesis is the recent observation that pharmacological protection of oligodendrocytes is beneficial in the clinical outcome of an experimental MS model.[346] Still, this challenging hypothesis awaits further research.

Further complicating the resolution of MS pathogenesis is the variety of lesion types seen throughout the brain of those afflicted with MS. While white matter lesions (WML) can be classified based on their immunological activity,[303–305] grey matter lesions (GML) are characterized by only minor infiltration of immune cells.[47,49] Whether WML and GML each represent a distinct type of pathology with a unique origin or sequential stages in the evolution of a single type of MS, has not yet been resolved. Although WML as well as GML are characterized by areas of focal demyelination, their histopathological features differ. The most prominent difference is the lower number of infiltrating immune cells in GML.[47,49] In this review, we summarize current knowledge on the pathological status of WML and GML and discuss cell types involved and several underlying factors possibly explaining the histopathological differences between these lesions.

WHITE AND GREY MATTER LESIONS IN MULTIPLE SCLEROSIS

White matter lesions

The location, size and shape of WML vary among patients. The anatomical localization of WML can explain certain neurological symptoms, such as visual dysfunction, which correlate with WML within the optic nerves and tracts within the left frontal lobe.[8,347] In addition, lesions within white matter tracts of the brainstem, e.g. the medial olivocochlear bundle, account for impaired auditory functioning.[11] Lesions within white matter tracts of the brainstem and cerebellum correlated with bowel and bladder dysfunction[202] and lesions within the corticospinal tract (e.g. the left internal capsule)[202] and sensory tract[259] within the spinal cord are most likely the underlying cause of motor and sensory deficits, respectively.[14,203]

Several ways of pathologically classifying white matter lesions have been described. The staging system defined by Bø and Trapp[303,304] distinguishes active, chronic active and inactive lesions. Active lesions are hypercellular and characterized by relative axonal preservation, massive infiltration of lymphocytes, MHC-II positive cells and/or myelin-laden macrophages that are evenly distributed throughout the lesion. Chronic active lesions also present with relative axonal preservation but the myelin-laden macrophages accumulate at the edges of the lesion. Inactive plaques are hypocellular lesions characterized by substantial loss of axons and oligodendrocytes, astrogliosis, and minor infiltration by macrophages/microglia and lymphocytes.[303–305,348] Another type of lesion, the preactive lesion, was added to this staging system. Preactive lesions are characterized by clusters of MHC-II positive microglia without demyelination and the absence of lymphocytes.[305,349–351]

An additional way of characterizing active WML is based on their pathological profiles, i.e. four distinct patterns of demyelination have been described. Pattern I is characterized by T cell infiltration, and active demyelination with many activated microglia and myelin-laden macrophages. However, no immunoglobulin (Ig) and complement deposition are present. Moreover, the demyelination process is characterized by a simultaneous loss of all myelin proteins from damaged myelin sheaths. Pattern II lesions, the most frequently seen pattern, is similar to pattern I, but additionally show Ig and complement deposition. Pattern III lesions also present with infiltrated inflammatory cells and microglia and macrophage activation, without Ig and complement deposition, but there is clear oligodendrocyte apoptosis, with a preferential loss of the protein myelin associated glycoprotein (MAG). Pattern IV lesions are extremely rare and are associated with non-apoptotic death of oligodendrocytes in a small rim of periplaque WM.[289,348] Whether these different patterns represent different subtypes of WM lesions or different stages within the formation of WM lesions is still debated.[342,348]

Grey matter lesions

Although MS was frequently considered a white matter disease and certain clinical deficits can be attributed to WML in functionally relevant tracts, the white matter did not always explain or predict the clinical symptoms and radiological observations in the patients [208].

This clinico-radiological paradox has been largely solved by accumulating evidence from histopathological[209,217,352] and high resolution imaging studies[46,211–214] showing that the CNS grey matter (GM) is also affected in MS patients. Moreover, the presence of GML was associated with clinical disability[46,212].

GML can occur in various brain regions of MS patients such as the cerebral cortex,[210,285,288] deep gray matter structures such as the thalamus[210,285] and the hippocampus,[142,144] the cerebellum[210,287] and the grey matter of the spinal cord.[210] The presence of these GML may explain certain cognitive impairments, e.g. lesions in the hippocampus and amygdala are associated with impaired memory[149,266] and psychiatric problems like depression correlated with temporal lobe lesions[353], which occur in a great number of MS patients already early in the disease.[188,193,194,215]

Until now three pathological patterns of GML have been described, but only for cortical demyelination.[49] Type I lesions are leukocortical lesions which include both subcortical white matter and cortex. Type II lesions are located within the cortex without extending to the surface of the brain or to the subcortical WM and type III lesions are subpial and extend from the pial surface into the cortex.[49] Bø et al. (2003) added a fourth category, type IV lesions, which extend throughout the full width of the cerebral cortex without affecting the white matter.[48] These lesions are not defined by their immunological activity.

THE CELLS INVOLVED IN MS PATHOLOGY

Infiltration of immune cells

The general view has been that MS pathology starts with the development of acute inflammatory lesions characterized by damage of the blood-brain barrier (BBB). This idea is mainly substantiated by the observation that active lesions are characterized by perivascular infiltration of leukocytes[354–357] and by data derived from animal models, notably experimental autoimmune encephalomyelitis (EAE).[120,290,291,358] Indeed, when peripheral monocytes and macrophages were depleted, clinical symptoms hardly developed in animals suffering from EAE.[292,359] The involvement of macrophages in the pathogenesis of MS is further substantiated by the observation that macrophages are present within WML of MS patients. Two phenotypes of macrophages have been identified, i.e. classically activated M1 macrophages and alternatively activated M2 macrophages. M1 macrophages typically express pro-inflammatory and cytotoxic factors that contribute to demyelination and axonal damage. M2 macrophages, on the other hand, contribute to a protective environment by secreting anti-inflammatory factors and growth factors.[360] Interestingly, in human *post mortem* active and chronic active WML activated macrophages were observed, the majority of which displayed an M1 activation status. However, a substantial part of the activated macrophages in these WML also expressed M2 characteristics, suggesting an intermediate activation state of macrophages in MS lesions.[361]

In addition to macrophages, different subsets of $CD4^+$ cells, T helper type 1 (Th1) and Th17 cells, and $CD8^+$ T-cells have been identified in EAE and MS lesions.[293,332–334] Studies in EAE show that $CD4^+$ T-cells become activated in the periphery before the onset of clinical symptoms of EAE.[71,331] However, accumulating evidence suggests an additional role for $CD8^+$ T-cells. This type of T-cell has been described to be cytotoxic and it has been proposed that lesion formation in EAE is initiated by $CD4^+$ T-cells, while amplification of the immune response and damage is mediated by $CD8^+$ T-cells.[291,332]

T-cells enter the CNS by passing the BBB and glia limitans.[362] The BBB is a selective functional barrier composed of endothelial cells, astrocyte endfeet, and pericytes. Between adjacent cerebral endothelial cells tight junctions are present. The three main tight junction family proteins are claudin, occludin, and junction adhesion molecules.[363,364] T-cells pass the BBB through a sequence of interactions with the brain endothelial cells, involving leukocyte rolling, adhesion, activation, arrest and eventually transendothelial migration. These complex interactions are depending on the interaction between L-selectin present on T-cells and E- and P-selectin present on inflamed endothelial cells and the expression of integrins and chemokines.[365] During MS, several factors, e.g. increased presence of inflammatory cytokines such as IL-17 and IL-22[366],can inflict damage to tight junctions. Once passing the glia limitans, regulated by macrophage-derived matrix metalloproteinases (MMPs)[367] into the CNS, T-cells are reactivated by local and infiltrating activated antigen-presenting cells (APC), which present self-antigens.[291,332] Subsequently, inflammatory processes lead to damaged myelin and axons.

T-cell infiltration and reactivity may not be the only crucial step during MS pathogenesis. When T-cell infiltration was prevented by using autologous haematopoietic stem cell transplantation, demyelination and axonal damage still occurred in regions with active macrophages and/or microglia.[368] In addition, cortical GML show significantly less infiltration of T-cells compared to WML.[47,49] The paucity of T-cells in GML questions their importance in GML formation. Thus, although T-cell infiltration appears to play a crucial role in the pathogenesis of MS, lesion formation can still occur in the absence of T-cells. In addition to T-cells, glial cells are known to be present in MS lesions, suggesting that glial cells also play an important role during lesion formation.

Activated microglia and astrocytes

Microglia are parenchymal tissue macrophages and the primary responding cells when the homeostasis of the brain is challenged by infection or injury.[54] Upon activation, microglia undergo a morphological transformation from a ramified to an amoeboid phenotype. They can either adapt a classically activated or M1 phenotype or an alternatively activated or M2 phenotype. M1 microglia are phagocytic and produce pro-inflammatory cytokines, e.g. interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, IL-12 and cytotoxic substances, e.g. nitric oxide (NO) and reactive oxygen species (ROS). M2 microglia are also phagocytic, but promote tissue regeneration by producing growth factors and/or anti-inflammatory cytokines,

e.g. transforming growth factor (TGF)-β, insulin-like growth factor (IGF)-1 and IL-10. M2 microglia are involved in downregulating the production of pro-inflammatory cytokines, increasing anti-inflammatory molecules and facilitating tissue repair[57,360,369]. In addition to microglia, astrocytes function as immunocompetent cells by secreting neurotrophic and/or neurotoxic factors[370,371] or augmenting the immune response by attracting immune cells from the blood circulation into the CNS.[372,373]

The exact role of glial cells in the pathogenesis of MS remains elusive. Studies in EAE animals suggest that their activation occurs in the early phase of lesion formation, even before infiltration of immune cells.[374–376] In addition, glial cells have been suggested to be involved in the attraction and migration of leukocytes towards sites of inflammation. Microglia and astrocyte signalling contributes to initiation and progression of lesion formation, and clinical symptoms in animal models of MS. Indeed, paralyzing microglia in CD11b-HSVTK transgenic mice [377] or preventing microglia signaling [378] resulted in a delay in disease onset and suppression of clinical signs in EAE. In addition, in a mouse model of MS using cuprizone-induced demyelination, specific ablation of astrocytes resulted in a significant reduction in demyelination in both white and grey matter, which was accompanied by a decrease in the number of activated microglia present at lesion sites.[379]

Despite the apparent detrimental role of glial cells in MS, a beneficial role of these cells has also been described. M2 microglia and/or macrophages and astrocytes are crucial for efficient remyelination in animal models of MS.[380–383] In addition, an intermediate microglia phenotype was recently described in both preactive and remyelinating MS lesions, i.e. microglia expressing M1 as well as M2 markers[384], which underlines the dual role of glial cells in MS.

Activated microglia

Activated microglia have been identified *post mortem* in WML and GML.[49,51] In addition, an *in vivo* study using positron emission tomography (PET) showed that activated microglia are present in cortical GM of MS patients.[385] Microglia present in human MS WML were found to express vascular cell adhesion molecule-1 (VCAM-1) which binds leukocytes.[386] Moreover, activated microglia are associated with lymphocyte and plasma cell infiltration of the meninges.[53] In addition to their involvement in the attraction of leukocytes through the BBB, several lines of evidence point towards a role for microglia in demyelination and axonal damage. For example, microglia are more located at the border of the lesion, the site where extensive oligodendrocyte damage occurs.[49] Recently, it was shown that activated microglia form perivascular clusters at sites of BBB leakage with fibrinogen deposition even before the onset of demyelination in EAE, which was associated with axonal damage.[387] In addition, an *in vitro*study showed that the expression of pro-inflammatory cytokines, inducible nitric oxide synthase (iNOS) and reactive oxygen species (ROS) by activated microglia results in a significant increase in damaged myelin and axons.[388] Activation of microglia is not only associated with tissue damage, but also with increased clinical disability of MS patients. A PET study using the peripheral benzodiazepine receptor ligand PK11195 showed a significant

increase in microglial activation in the cortical GM of patients with RRMS or SPMS, which correlated with clinical disability.[385] Moreover, immunohistochemical data of MS autopsy cortex tissue showed less favourable disease course when cortical lesions presented with a rim of activated microglia at their border.[216] In general, less activated microglial cells are present in GML compared to WML. Of interest is a recent study that showed that MS patients having the HLA-DRB1*1501 genotype present with increased microglial activation within cortical lesions than MS patients without this genotype,[389] suggesting a genotype-specific component contributing to microglial activation in, at least, GML.

In contrast to a detrimental clinical outcome associated with microglia activation, a protective role of microglia has also been described (reviewed in Napoli and Neumann (2010)[390]). Microglia in the normal appearing white matter (NAWM) of MS patients have an alerted but immunosuppressed phenotype, suggesting a protective role for microglia in the early phase of lesion formation.[391] During the active phase of demyelination, amoeboid microglia containing ingested myelin-derived lipids have been shown to produce anti-inflammatory cytokines and growth factors that promote regeneration.[70] Moreover, phagocytic microglia have been observed in MS lesions containing the triggering receptor expressed on myeloid cells-2 (TREM-2). The TREM-2 receptor plays a crucial role in the process of myelin debris clearance.[392] Blocking TREM-2 in EAE results in more severe EAE, characterized by higher clinical scores.[393] In addition, since myelin debris is an inhibitor of remyelination, removal of myelin debris is essential for optimal remyelination.[394]

Activated astrocytes

Not only activated microglia, but also hypertrophic astrocytes and astrogliosis have been identified in WML, and to a lesser extent in GML, of MS patients.[52] In addition to their ability to provide metabolic support for neurons, taking up and releasing neurotransmitters, and maintaining blood-brain barrier function, astrocytes have also been described as immunocompetent cells. Astrocytes express various pattern recognition receptors (PRR), e.g. Toll like receptors (TLRs). Upon activation, astrocytes contribute to the immune response of the CNS, either by secreting pro-inflammatory cytokines or neurotrophic factors, e.g. brain-derived neurotrophic factor (BDNF).[371] In addition, astrocytes are able to augment the immune response by attracting immune cells from the blood circulation into the CNS, either by increasing BBB permeability or via the release of chemokines, e.g. monocyte chemoattractant protein-1 (MCP-1, also known as CCL2).[372,373] In a later stage of MS, astrocytes become more helpful by promoting remyelination and tissue repair.[383] On the contrary, scar formation by astrocytes is known to inhibit remyelination by preventing the migration of oligodendrocyte precursor cells (OPC's) towards the lesion.[395]

To conclude, both activated microglia and astrocytes are present in WML and, to a lesser extent, in GML, The exact functions that microglia and astrocytes are exerting are depending on the timing of their activation and the subsequent production of pro- or anti-inflammatory factors, which can either create conditions that can be detrimental and lead to demyelination or an environment which promotes remyelination and prevents axonal loss.

Pathological cellular differences between WM and GM lesions

Although studies on biopsy material showed infiltrated leukocytes within early GML,[217,294] several histopathological post-mortem studies demonstrated that infiltrated leukocytes are far less present within GML than within WML.

Immunohistochemical analysis of $CD3^+$ T-cells and $CD68^+$ microglia and/or macrophages in WML significantly outnumbered the number of these cells observed in cortical GML of MS patients. This observation is best illustrated in leukocortical lesions encompassing WM and GM (type I lesions) where WM areas encompass higher levels of T-cells and microglia/macrophages and GM areas are almost devoid of these inflammatory cells.[49] In addition, the WM part of type I lesions presented with a significantly higher number

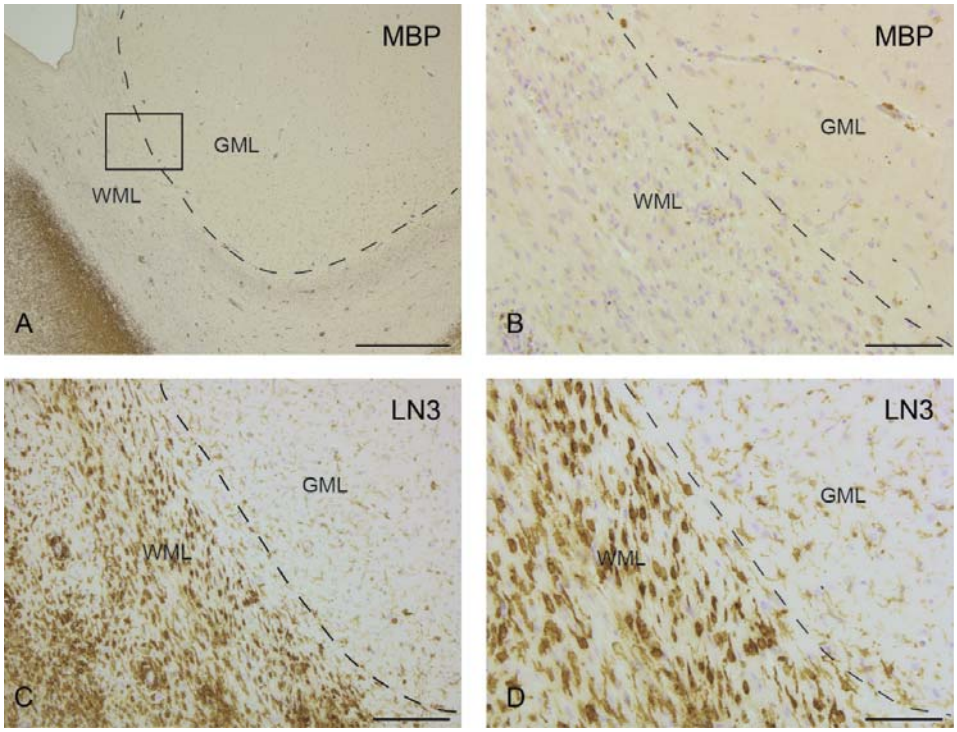

Figure 1. MHC-II positive cells in a mixed hippocampal WM/GM lesion. MS lesions are recognized by the loss of myelin basic protein (MBP) immunoreactivity in WM and GM areas. (**A**) Loss of MBP immunoreactivity in a hippocampal lesion of a MS patient. Some MBP immunoreactivity is still present in the non-lesioned area (lower left corner). (**B**) A higher magnification clearly shows the absence of MBP immunoreactivity in WM and GM lesion area. (**C**) The WM part of the lesion is characterized by the numerous presence of MHC-II positive cells, while in the GM part of the lesion less MHC-II positive cells can be found. (**D**) At a higher magnification, it becomes clear that the MHC-II positive cells in the WML have mostly an amoeboid shape, whereas in the GML they represent with a more ramified morphology. The dotted line indicates the border between WML and GML. The frame in A refers to the areas shown in (**B**-**D**). Scale bar (**A**) = 1 mm; scalebar (**B**,**D**) = 50 μm; scalebar (**C**) = 100 μm. For detailed description of immunohistological methods, we refer to Prins et al., 2014.[397]

of LN3$^+$ and CD68$^+$ cells compared to the GM part of the same type I lesion,[396] which was also observed in hippocampal lesions concerning LN3$^+$ cells (see figure 1).[397]

Moreover, numbers of CD3$^+$ T-cells were significantly lower in intracortical lesions compared to type I lesions.[47] Similarly, CD8$^+$, CD4$^+$ and CD45RO$^+$ T-cells were most frequently detected in WML, less in type I lesions and the lowest number of these cells was observed in intracortical lesions.[47] Interestingly, deep grey matter (DGM) lesions are characterised by an intermediate inflammatory phenotype, as the number of CD3$^+$ T-cells in this type of lesion was significantly higher compared to control DGM, although not as high as in WML.[398] Similarly, *in vivo* microglia activation in DGM, e.g. the thalamus, was higher compared to cortical areas but lower compared to WM areas in patients suffering from clinical isolated syndrome (CIS), a first clinical symptom suggestive of MS.[399]

EXPLANATIONS FOR PATHOLOGICAL CELLULAR DIFFERENCES BETWEEN WML AND GML

The underlying mechanisms resulting in the apparent pathological cellular differences between WML and GML remain to be explained. Thus far, several options have come into focus, such as temporal, local, cellular, and molecular differences in the WM compared to the GM (summarized in table 1), that ultimately, however, still result in a similar outcome, i.e. demyelinating activity and formation of WML and GML.

Temporal difference between presence of inflammatory cells in WML vs GML

Although a study on cortical demyelination using an immunohistochemical approach on autopsy material found that demyelination in the cerebral cortex was mostly seen in MS patients in the chronic progressive phases of the disease and only rarely in patients in the acute or relapsing phase[209], several studies have now reported that GML are present already during the early stages of MS, even when WM pathology is very limited.[46,400] Moreover, GM demyelination was recently reported in early active MS using an immunohistochemical approach on biopsy material.[217] This finding is supported by a case study showing demyelination in biopsy material of an early MS patient, even before radiological evidence of WMLs became visible on MRI scans.[294] Although no comparison was done with WML in biopsy material, infiltrating CD3$^+$ and CD8$^+$ T-cells were frequently present in this biopsy material of cortical lesions[217,294] as can be observed in *post mortem* WML. In contrast, immunohistochemical studies showed a paucity of infiltrating immune cells in *post mortem* GM cortical and hippocampal lesions.[47,49,397] This contrast in immunopathology of post-mortem WML and GML warrants further research. However, we cannot exclude the possibility that lesions found in biopsy material are in an earlier stage of demyelination compared to lesions in *post mortem* tissue and that infiltrating immune cells are only present during the early stages of GML formation.

Other T-cell entry sites

BBB damage is one of the main characteristics of white matter lesions in MS. This, together with passage of the glia limitans gives T-cells access to the CNS. Thusfar, no blood-brain-barrier leakage has been demonstrated in GML[325] and little or no significant complement deposition in GML was observed[295,401]. Additionally, the diameter of the blood vessels within the lesions was found to be increased in WML, whereas this increase was less within GML.[402]

Instead of infiltration of immune cells through the BBB, several studies have suggested a causal link between meningeal inflammation and cortical GM demyelination.[335,336] In addition, a significant interdependence between the presence of activated microglia and meningeal T-cell infiltration was observed, suggesting that microglial activation in the cortex of MS patients is, at least in part, driven by the meningeal inflammatory response.[53]

Although this may hold true for cortical lesions in MS, this does not necessarily explain the occurrence of subcortical GM lesions, e.g. hippocampal lesions or lesions within the thalamus. Another pathway through which T-cells can enter the CNS is via the choroid plexus (CP).[235,403] A recent study showed that the CP is populated by a distinct population of $CD4^+$ T cells, which differ from those in the blood circulation, with T-cell receptors specific to CNS antigens.[404] Of interest is the observation that during EAE, CCR6 positive T-cells enter the CNS via the CP via a CCL20 dependent pathway.[405]

Thus, BBB damage was not detected within GML, however T-cells can also enter the CNS via de meninges or the CP. Nevertheless, deep grey matter structure that are not directly located next to meninges or the CP probably lack T-cell infiltration via these routes.

Local differences

Immunosuppressive properties of neurons

In contrast to the WM, which is devoid of neurons but has numerous oligodendrocytes in addition to microglia and astroglia, the GM is mainly composed of neurons surrounded by GM astrocytes and microglia and less oligodendrocytes. The presence of neurons, might be causing the quick resolution of inflammation in GML by creating an anti-inflammatory environment. Neurons are able to inhibit immune responses by constitutively expressing a wide array of membrane-bound molecules, e.g. CD200 and CD47 and by releasing factors, e.g. chemokines and neurotransmitters.[322,406] The CD200 receptor (CD200R) is expressed on macrophages, microglia and T-cells. Upon CD200R-CD200 signalling, the production of pro-inflammatory cytokines by primary microglia, e.g. interleukin-1β and IL-6 is decreased while the production of the anti-inflammatory cytokine IL-10 is increased in a microglia-neuron co-culture system. This IL-10 protects neurons from inflammatory damage[407] suggesting that the expression and activation of the CD200R on macrophages/microglia by neuronal CD200 is anti-inflammatory and neuroprotective. In addition, CD47 expressed by neurons interacts with its receptor signal-regulatory protein-α (SIRP-α) present on microglia and/or macrophages and inhibits TNF-α expression and phagocytosis.[408,409] However, CD200[410] and CD47[411] are

also known to be expressed by oligodendrocytes, exerting the same immunosuppressive function within the WM. Interestingly, CD200 and CD47 expression is significantly reduced in and around WML[412], suggesting a reduced immunosuppressive environment within WML. However, whether CD200 and CD47 are also down-regulated within GML remains to be determined. One membrane-bound molecule that is exclusively expressed by neurons is the neuronal cell adhesion molecule ICAM-5, which down regulates T-cell activation.[413] In addition to membrane-bound molecules, neurons secrete several factors, e.g. chemokines and neurotransmitters. Neurons constitutively express CX3CL1 which interacts with the CX3CR1 receptor present on microglia[414] and T-cells[415], thereby suppressing the production of pro-inflammatory cytokines and nitric oxide (NO) and neuronal cell death induced by activated microglia.[416,417]

Table 1: Several immunopathological characteristics of WML and GML in post-mortem material of MS patients

	WML	GML
Cell influx	Infiltrating T-cells and macrophages observed by *post mortem* immunohistopathological analysis[293,332–334,354–357,361]	Little or no inflammatory cells present as observed by *post mortem* immunohistopathological analysis[47,49,397] , but observed in biopsy material[217,294].
Local characteristics	Myelin debris inducing an inflammatory response[396,423]	Neuronal dampening of the immune response by membrane bound molecules[322,406] and by secreting neurotransmitters[419,420]
T-cell site of entry	Blood-brain-barrier[289]	Meninges[335,336] or choroid plexus[232,405]
Glial cell characteristics		
• *Number of microglia*	• Higher than in GM[326]	• Lower than in WM[326]
• *Inflammatory profile of microglia*	• More prone to be pro-inflammatory[327,328], complement deposition[295]	• Less prone to be pro-inflammatory[327,328], no complement deposition[295]
• *Expression of molecules that regulate leukocyte migration*	• High microglial MRP-14[426] and astrocytic CCL-2 expression[317,397]	• Little microglial MRP-14[426] and astrocytic CCL-2 expression[317,397]

Neurotransmitters

The two major neurotransmitters in the brain, glutamate and GABA, are not only functional neurotransmitters, but also act as immunomodulators. The effects of glutamate on immune activity are dual. Several glutamate receptors have been described, i.e. ionotropic and metabotropic receptors.[164,418] Metabotropic glutamate receptors (mGLuRs) consist of three subgroups. Depending on which group of mGLuRs is activated, glutamate can be immunosuppressive as well as stimulate the release of pro-inflammatory cytokines. By the activation of group III mGLuRs glutamate attenuates the neurotoxicity of microglia[419], while activation of group II mGLuRs can induce TNF-α expression by microglia.[420] Since TNF-α, among other pro-inflammatory factors, is involved in the influx of immune cells through the BBB, glutamate can indirectly affect this event during MS. Glutamate can exert its effects in WM as well as GM, since glutamate can be released within the synaptic cleft, but axonal release within the WM is also possible.[421] However, GABA levels are significantly higher within human cortical grey matter compared to white matter.[422] GABA is known for its immunosuppressive effects, e.g. IL-6 and IL-20p40 release by microglia is attenuated by GABA[420], suggesting that GABA contributes to a more immunosuppressive environment within the GM than in the WM. Thus, GABA indirectly reduces immune cell infiltration by inducing immunosuppressive factors.

Myelin induced immune reaction

The WM is mainly composed of axons enwrapped with myelin, formed by oligodendrocytes. A higher amount of myelin within the WM than the GM, results in more myelin debris in WML than in GML during MS lesions formation. Thus, the increased presence of myelin debris could contribute to the significant higher numbers of activated immune cells within WML. A histopathological study in cuprizone induced demyelination showed that lesions in GM areas with inherently lower levels of myelin present with less activated microglia and astrogliosis compared to GM areas with a higher density of myelin or WM areas[396,423], while there is no evidence of a difference between the total numbers of glial cells in WM and GM in mice.[396] Similar results were observed in type I leukocortical lesions of MS patients, i.e. the myelin rich cortical layer showed more $LN3^+$ cells compared to other cortical layers encompassing less myelin.[396] Moreover, when WM and GM areas in the mouse brain were injected with similar amounts of myelin-debris, both areas presented with a similar number of activated microglia and astrocytes.[396] This suggests that the amount of myelin debris, which is higher in WM compared to GM, is a crucial factor for activation of local immune cells, i.e. glia.

Glial cell differences in white and grey matter

Besides the observed difference in infiltrating T-cells in WM compared to GM lesions, a difference between these types of lesions concerning activated glial cells was observed as well. This questions whether glial cells in WM and GM differ. In humans, microglial cells

in WM exceed those in GM,[326] although this is the reverse in rodents where more microglia can be found in GM than in WM.[424] In addition, a constitutively higher number of HLA-DR positive microglia has been found in WM than in GM *post mortem* normal human brain tissue.[327] Moreover, a recent study showed that WM microglia showed more age-related phenotypic changes than GM microglia, e.g. increased expression of functional markers such as CD68, F4/80 and CD11 was shown, specifically in white matter of aged mice.[328] suggesting a higher reactivity to disturbed homeostasis within WM compared to GM. We consider of importance the local differences in molecules involved in leukocyte attraction and migration. The expression of the macrophage early activation marker migration inhibitory factor-related protein-14 (MRP-14), known to regulate leukocyte migration,[425] by perivascular macrophages and/or microglial cells was found to be significantly less expressed in the neocortex compared to white matter in the marmoset EAE model of MS.[426] Moreover, astrocytic expression of CCL2, known for its role in the process of attracting peripheral immune cells, was found to be clearly expressed in WML whereas hardly present in GML of MS patients[397] and the cuprizone mouse model of MS.[317] Of interest is that the receptor for CCL2, i.e. CCR2 was present on microglia in both WML and GML (see figure 2).[397] These observations may explain the pathological difference found between WML and GML, in that a factor of importance for attracting immune cells, i.e. CCL2, is hardly present in GML, resulting in the absence of those cells in GML in contrast to WML where CCL2 is abundantly expressed in MS.

CONCLUSION

For a long time MS was considered to a disease of the WM in the CNS driven by T-cells which are reactive against myelin-antigens resulting in demyelination, oligodendrocyte death and axonal damage. However, more recent imaging and immunohistochemical observations recognize the formation of lesions in GM as an important pathological, and clinical relevant process as well. In the present review, we focus on the observed pathological difference between WML and GML. The low number of leukocytes in GML implies that either demyelination does not require the presence of leukocytes or that the pathogenesis of WML formation differs from that of GML. Evidence already points towards a possible difference in the pathways underlying demyelination in WM and GM, such as the finding that there is no, or only low, correlation between the extent of demyelination in the WML and GML.[209,285,352]. We now put forward that cellular and molecular differences residing within glial cell populations in WM and GM, in particular molecules expressed by them, and the local cellular environment determine the pathway to demyelination which in GML formation is, largely, irrespective of immune cell infiltration. Future studies should focus on further understanding of the differences between WML and GML formation. In our view, increased understanding of GML pathogenesis which is considered to underlie disability in chronic MS may have direct impact on the development of novel therapeutic targets to counteract this progressive neurological disorder.

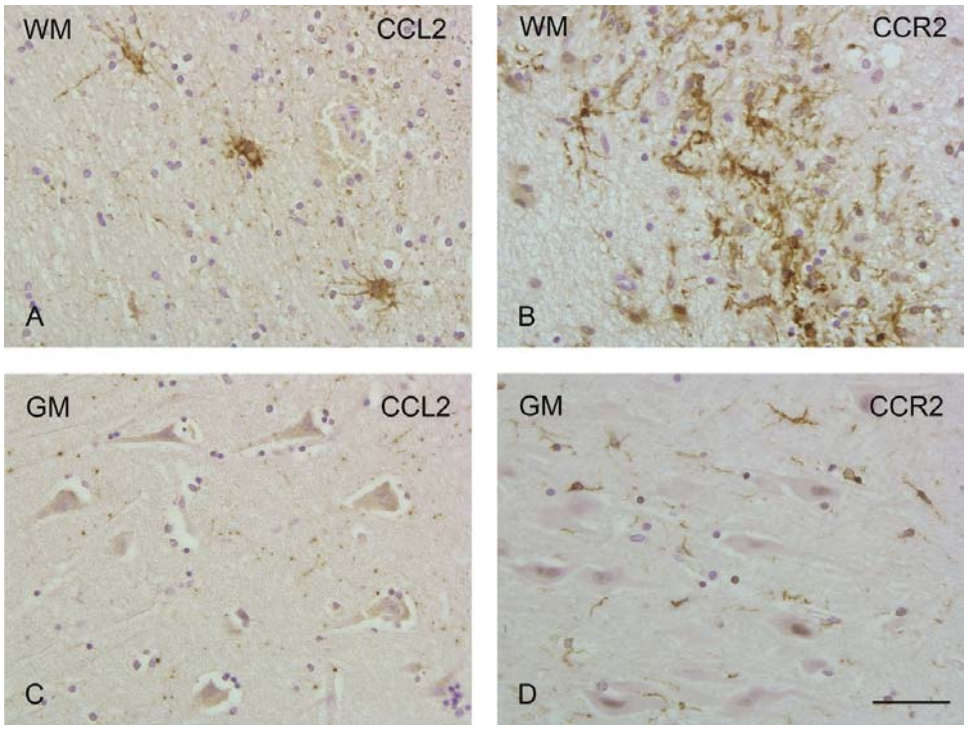

Figure 2. CCL2 and CCR2 immunoreactivity in WM and GM bordering a hippocampal lesion. (**A**) WM bordering a lesion shows CCL2 immunopositive cells and (**B**) numerous CCR2 immunopositive cells. In contrast, (**C**) GM bordering the same lesion is devoid of CCL2 expressing cells, but it does (**D**) show CCR2 immunoreactivity, although to a lesser extent compared to WM. Scale bar (**A**-**D**) = 25 μm. For detailed description of immunohistochemical methods, we refer to Prins et al., 2014.[397]