"INSIDE-OUT" VS "OUTSIDE-IN" PARADIGMS IN MULTIPLE SCLEROSIS ETIOPATHOGENESIS

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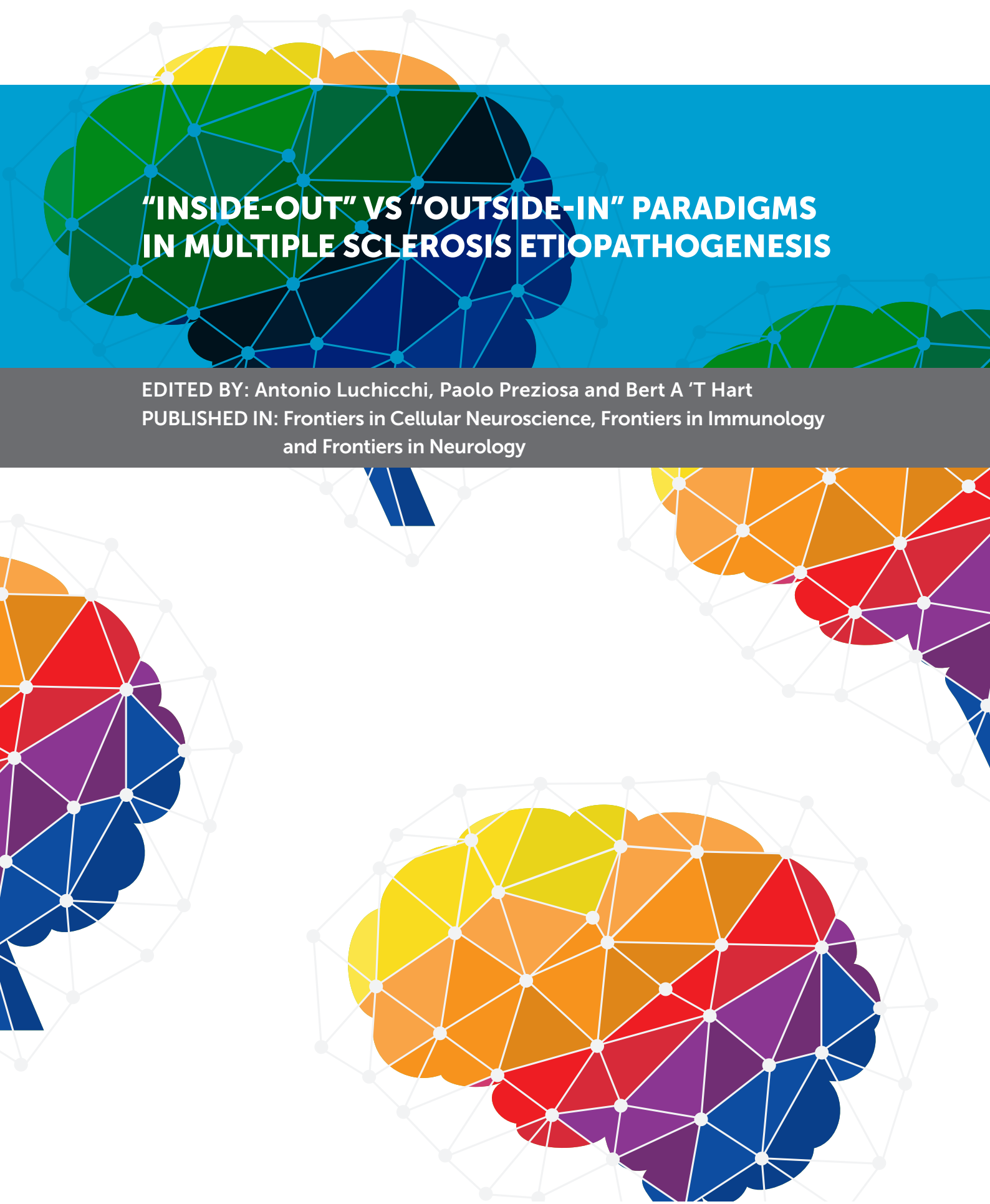

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"INSIDE-OUT" VS "OUTSIDE-IN" PARADIGMS IN MULTIPLE SCLEROSIS ETIOPATHOGENESIS

Topic Editors:
Antonio Luchicchi, VU University Medical Center, Netherlands
Paolo Preziosa, Vita-Salute San Raffaele University, Italy
Bert A 'T Hart, University Medical Center Groningen, Netherlands

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EDITORIAL
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Editorial: "Inside-Out" vs "Outside-In" Paradigms in Multiple Sclerosis Etiopathogenesis

Antonio Luchicchi[1]*, *Paolo Preziosa*[2,3] *and Bert 't Hart*[1]

[1] Division Clinical Neurosciences, Department of Anatomy and Neurosciences, Amsterdam Universitair Medische Centra, Vrije Universiteit Medical Center, Amsterdam, Netherlands, [2] Neuroimaging Research Unit, Division of Neuroscience, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy, [3] Neurology Unit, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy

Keywords: multiple sclerosis, pathogenesis, outside-in, animal models, biomarkers, molecular aspects, inside-out

Editorial on the Research Topic

"Inside-Out" vs "Outside-In" Paradigms in Multiple Sclerosis Etiopathogenesis

Multiple sclerosis (MS) is a chronic inflammatory/demyelinating disease of the central nervous system (CNS) (Filippi et al., 2018) of unknown etiology.

Two complementary paradigms, indicated as "*outside-in*" and the "*inside-out,*" are leading discussions about the MS origin (Stys et al., 2012). The outside-in paradigm posits a peripherally-elicited autoimmune attack against myelin as root cause for MS, whereas inside-out implicates a primary CNS cytodegenerative process alarming secondary autoimmune reactions against myelin debris.

Which of the two theories better depicts the very first pathological changes in MS is debated. Since a better evaluation of MS etiopathogenesis could also optimize patients' management, the present issue was aimed to stimulate a discussion about possible overlooked candidates in support of each theory. We collected nine contributions where authors discuss the *outside-in*/*inside-out* paradigms from different angles.

Three main aspects were discussed: (i) animal models of MS etiopathology, (ii) cellular processes in MS pathophysiology, and (iii) tools and biomarkers enabling investigation of *in vivo* pathophysiological mechanisms already from the prodromic/early phases of MS.

Titus et al. and Sen et al. summarized the most updated models supporting either the *outside-in* or the *inside-out* theory of MS. In particular, the first study concluded that, due to the heterogeneous manifestation of MS pathology, a combination of both paradigms, rather than one of the two, may better explain the origin of MS. Conversely, the second article supported the value of more recently developed models, like the cuprizone (CPZ) mouse, to investigate primitive changes occurring in MS brains. Interestingly, CPZ models seem ideal to reveal the mechanisms involved in MS origin before the immune attack intervenes, and to study MS progression in conditions of immune reaction and protection.

It is well-known that several genetic factors contribute to the increased risk to develop MS (International Multiple Sclerosis Genetics Consortium, 2019). In their opinion article, Ferrè et al. provided an update of genetic factors associated with MS onset, progression and treatment response. At present, the majority of genes and biological processes associated with a higher MS risk are implicated in immune functions (International Multiple Sclerosis Genetics Consortium, 2019). Conversely, only a few genetic loci involved in oligodendrocyte maturation have been suggested to contribute to MS occurrence (Factor et al., 2020). Accordingly, dysregulation of immune responses, promoted by the genetic background and potentially triggered by environmental factors, could represent the main mechanism in MS onset and progression.

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Dirk M. Hermann, University of Duisburg-Essen, Germany

***Correspondence:**
Antonio Luchicchi
a.luchicchi@amsterdamumc.nl

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Of the variety of molecular pathways involved in MS origin, several emphasize a central role of the immune system. Misrielal et al. review the role of autophagy, whereas Morgan et al. underline the role of the complement system in MS pathophysiology. The novelty resides in the completely renewed look that authors gave to these two processes in MS pathogenesis. Both autophagy and complement production could be equally supportive of either a primary immune attack against myelin or a primary response to a CNS cytodegenerative process, paving the way for future experiments that can potentially unveil the real cause of MS.

Interestingly, both autophagy and complement production may hold consequences for the stability of mitochondria, whose role in MS is not new (Witte et al., 2014). Recent attention has been dedicated to these organelles due to their putative involvement in the destabilization of the newly described axon-myelinic synapse (AMS), a dynamic form of communication between axon and myelin whose malfunction has been proposed to explain the origin of different neurodegenerative conditions (Micu et al., 2018), including MS (Luchicchi et al., 2021). In line with this notion, two articles by Bergaglio et al. and Poertoawmodjo et al. focused on the role of mitochondria instability as primitive trigger of MS pathology from two different angles: the role of oxidative stress-derived mitochondrial impairment and the possible interplay between mitochondria and Ca^{2+}-dependent cysteine proteases.

Pathological studies and experimental models are the gold standard approaches to investigate MS pathophysiology. However, recently, several laboratory, neuroradiological and neurophysiological tools have been applied to define reliable biomarkers for understanding MS development and progression *in vivo*. Promising biomarkers have been presented in the two original research articles of our collection. Cennamo et al. applied Optical Coherence Tomography to investigate the role of peripapillary vessel density as an early biomarker in MS. This tool was relevant for identifying patients in the earliest phases of MS. Todea et al. showed how magnetic resonance imaging (MRI) enables identification of focal demyelination in white matter and cortical lesions already from the earliest phases of MS. Lesion burden was associated with serum neurofilament light chain (sNfL) levels, a biomarker of axonal injury. Moreover, the 2-year longitudinal changes of cortical and white matter lesion burden correlated with the cognitive performances of MS patients. This underlined the relevance of MRI biomarkers and sNfL to identify from the earliest disease phases reliable markers of neurodegeneration, disease severity and progression.

In conclusion, this issue of *Frontiers in Cellular Neuroscience*, *-Immunology,* and *-Neurology* provides an integrated overview of the "*hot topics*" in the field of MS cause. Emerging from the article collection is a complex picture where a dichotomy between outside-in/inside-out theories is replaced by a more integrated vision where both theories might equally apply according to the specific condition. The studies published in this issue emphasize the need for a re-evaluation of cellular processes, previously regarded as pure indicators of immune attack (e.g., complement), in combination with the individual (genetic) variability of MS patients, and the development of highly predictive experimental models/accurate biomarkers to unravel the unknown cause of the MS in the coming years.

AUTHOR CONTRIBUTIONS

AL, PP, and B'tH wrote the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

Factor, D. C., Barbeau, A. M., Allan, K. C., Hu, L. R., Madhavan, M., Hoang, A. T., et al. (2020). Cell type-specific intralocus interactions reveal oligodendrocyte mechanisms in MS. *Cell* 181, 382–395 e321. doi: 10.1016/j.cell.2020.03.002

Filippi, M., Bar-Or, A., Piehl, F., Preziosa, P., Solari, A., Vukusic, S., et al. (2018). Multiple sclerosis. *Nat. Rev. Dis. Primers* 4:43. doi: 10.1038/s41572-018-0041-4

International Multiple Sclerosis Genetics Consortium (2019). Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science* 365: eaav7188. doi: 10.1126/science.aav7188

Luchicchi, A., Hart, B., Frigerio, I., Van Dam, A. M., Perna, L., Offerhaus, H. L., et al. (2021). Axon-myelin unit blistering as early event in MS normal appearing white matter. *Ann. Neurol*. doi: 10.1002/ana.26014. [Epub ahead of print].

Micu, I., Plemel, J. R., Caprariello, A. V., Nave, K. A., and Stys, P. K. (2018). Axo-myelinic neurotransmission: a novel mode of cell signalling in the central nervous system. *Nat. Rev. Neurosci.* 19, 49–58. doi: 10.1038/nrn.2017.128

Stys, P. K., Zamponi, G. W., Van Minnen, J., and Geurts, J. J. (2012). Will the real multiple sclerosis please stand up? *Nat. Rev. Neurosci.* 13, 507–514. doi: 10.1038/nrn3275

Witte, M. E., Mahad, D. J., Lassmann, H., and Van Horssen, J. (2014). Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. *Trends Mol. Med.* 20, 179–187. doi: 10.1016/j.molmed.2013.11.007

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Peripapillary Vessel Density as Early Biomarker in Multiple Sclerosis

Gilda Cennamo[1†], Antonio Carotenuto[2†], Daniela Montorio[2], Maria Petracca[2], Marcello Moccia[2], Antonietta Melenzane[2], Fausto Tranfa[2], Anna Lamberti[2], Antonio L. Spiezia[2], Giuseppe Servillo[2], Marcello De Angelis[2], Martina Petruzzo[2], Chiara Criscuolo[2], Roberta Lanzillo[2‡] and Vincenzo Brescia Morra[2‡]*

[1] Eye Clinic, Public Health Department, University of Naples Federico II, Naples, Italy, [2] Department of Neurosciences, Reproductive and Odontostomatological Sciences, Federico II University, Naples, Italy

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Edited by:
Paolo Preziosa, Vita-Salute San Raffaele University, Italy

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Friedemann Paul, Charité—Universitätsmedizin Berlin, Germany
Letizia Leocani, San Raffaele Hospital (IRCCS), Italy

****Correspondence:***
Roberta Lanzillo
robertalanzillo@libero.it

†These authors share first authorship

‡These authors share senior authorship

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Background: To evaluate retinal vessel density (VD) in macular and in peripapillary regions in patients with recent onset of multiple sclerosis, at initial demyelinating event (IDE) and in matched relapsing-remitting multiple sclerosis (RRMS) patients.

Methods: We evaluated VD in superficial capillary plexus, deep capillary plexus, choriocapillaris and radial peripapillary capillary plexus in IDE, RRMS patients and in matched healthy controls (HCs) through Optical Coherence Tomography Angiography (OCT-A). Clinical history, including history of optic neuritis, Expanded Disability Status scale and disease duration of patients were collected.

Results: Thirty patients (20 with IDE and 10 with RRMS) and 15 HCs were enrolled. IDE patients showed a lower VD in radial peripapillary capillary plexus compared with controls (coeff. $\beta = -3.578$; $p = 0.002$). RRMS patients displayed a lower VD in both superficial capillary plexus and radial peripapillary capillary plexus compared with HCs (coeff. $\beta = -4.955$; $p = 0.002$, and coeff. $\beta = -7.446$; $p < 0.001$, respectively). Furthermore, RRMS patients showed a decreased VD in radial peripapillary capillary plexus compared with IDE patients (coeff. $\beta = -3.868$; $p = 0.003$).

Conclusions: Peripapillary region vessel density reduction, revealed through OCT-A, might be considered as an early event in MS, and might be relevant as a biomarker of disease pathology.

Keywords: vascular pathology, biomarker, angio-optical coherence tomography, clinically isolated syndrome, vessel density (VD)

INTRODUCTION

Multiple sclerosis (MS) is characterized by inflammation, demyelination and axonal loss throughout the central nervous system. Markers for disease pathology are highly needed. The introduction of the optical coherence tomography (OCT), fast and non-invasive imaging technique, allowed to investigate and monitor the structural retinal damages, in particular the ganglion cell and retinal nerve fiber layers in neurological diseases. Previous studies, analyzing the different disease stages often accompanied by optic neuritis, demonstrated that the retinal changes reflect not only the neurodegenerative processes but also the inflammatory disease activity.

Since the strong association described between the retinal impairment and brain atrophy on MRI, the OCT parameters play a significant role as biomarkers for MS diagnosis and follow up (1–3). Several reports also described cerebral hypo-perfusion and vascular pathology as pathological changes underpinning MS etiology and evolution (4, 5). Besides the application of advanced MRI techniques such as arterial spin-labeling, optical coherence tomography angiography (OCT-A) offers the unique opportunity to assess integrity of brain vasculature by looking at vascular networks within the retina (6, 7). We recently described a reduction of retinal vessel density (VD) on OCT-A in MS compared with controls. Reduced VD was associated with higher disability, as measured with Expanded Disability Status Scale (EDSS) (8), and was further confirmed over the 1-year follow-up, suggesting VD is a marker of disability, with improved vascularization being inversely associated with lower disability accrual. However, the inclusion of very early cases of MS would have allowed a better understanding about implication of retinal vasculature changes in the disease pathogenesis. Feucht et al. recently studied retinal vasculature network in patients with clinically isolated syndrome (CIS), and found vessel rarefaction of superficial and deep retinal plexus only in eyes suffering from previous optic neuritis, while a higher VD in choriocapillaris layer was associated with recent relapses and MRI activity (9).

The aim of this study is to investigate VD in macular and peripapillary regions in patients with an initial demyelinating event (IDE), namely patients experiencing the first neurologic symptom referable to demyelination in the central nervous system regardless they meet MS or CIS diagnosis at MRI scan according with 2017 McDonald criteria (10). We also aimed to compare vascular changes in the retina between controls, IDE and relapsing-remitting MS (RR-MS) patients through OCT-A.

METHODS

In this cross-sectional study, we enrolled IDE patients and healthy controls (HCs) at the MS Center of the University of Naples "Federico II," from January 2018 to June 2019. "IDE (initial demyelinating event) was defined as the first neurologic symptom referable to demyelination in the central nervous system, lasting for at least 48 h, regardless patients met RR-MS or CIS diagnosis according with 2017 McDonald criteria (10). We excluded patients with any history of optic neuritis, in order to avoid a bias related to optic nerve direct damage. Family history, motor disability assessed through EDSS, disease duration and previous relapses were recorded for all patients. HC presented with normal neurological and ophthalmic examinations.

Exclusion criteria were (i) a relapse and/or corticosteroid use in the previous month (ii) the presence of systemic vascular diseases (high blood pressure, diabetes, and heart diseases), (iii) clinically relevant lens opacities, (iv) low-quality images obtained with Spectral Domain (SD)-OCT and OCT-A, (v) myopia >6 diopters, (vi) history of intraocular surgery, vitreoretinal, and retinal vascular diseases, uveitis, congenital eye disorders.

Each subject underwent evaluation of best-corrected visual acuity according to the Early Treatment of Diabetic Retinopathy Study (11), slit-lamp biomicroscopy, fundus examination For each subject, we also assessed the mean deviation and pattern standard deviation as measures of visual field for subject with visual fixation above 20%. Finally, we performed both SD-OCT and OCT-A. Ophthalmological evaluation was blinded to subjects' clinical status. The study was approved by the Institutional Review Board of the University of Naples "Federico II" and all investigations adhered to the tenets of the Declaration of Helsinki (protocol number: 142/19). Written informed consents were obtained from the subjects enrolled in the study. The data that support the findings of this study are available from the corresponding author upon reasonable request.

SD-OCT

The retinal nerve fiber layer and ganglion cell complex thickness were obtained with SD-OCT (software RTVue XR version 2017.1.0.151, Optovue Inc., Fremont, CA, USA). The acquisition protocol for optic nerve head map was used to calculate the retinal nerve fiber layer thickness and it was based on measurements around a circle 3.45 mm in diameter centered on the optic disc. The ganglion cell complex thickness was obtained centering the scan 1-mm temporal to the fovea and covering a 7 × 7 mm area over the macular region. The ganglion cell complex thickness included the measurements from the internal limiting membrane to the outer boundary of the inner plexiform layer (12). Each OCT scan was analyzed in alignment following APOSTEL recommendations and applying the OSCAR-IB protocol for quality control (13, 14). These guidelines were adapted for our device.

OCT-A

OCT-A images were performed using the RTVue XR Avanti, Optovue, Inc. (software RTVue XR version 2017.1.0.151 Freemont, California, USA) following a standardized protocol based on the split-spectrum amplitude de-correlation algorithm, as previously described (15). Macular capillary plexus was visualized performing a 6 × 6 mm scan over the macular region and the percentage area occupied by the large vessels and microvasculature in the analyzed region defined the vessel density (16). The software identified the VD in whole area of the macular scan considering the two retinal vascular networks, namely the superficial and deep capillary plexuses, and choriocapillaris. The Angio-Vue disc mode automatically segmented the radial peripapillary capillary plexus VD analyzing the whole papillary region with a scanning area of 4.5 × 4.5 mm. VD for the radial peripapillary capillary plexus was analyzed in the superficial retinal layers and extended from the inner layer membrane to the retinal nerve fiber layer posterior boundary (17). From the analysis were excluded the images with a signal strength index <40 and residual motion artifacts. A summary of measures evaluated through SD-OCT and OCT-A is reported in **Figure 1**.

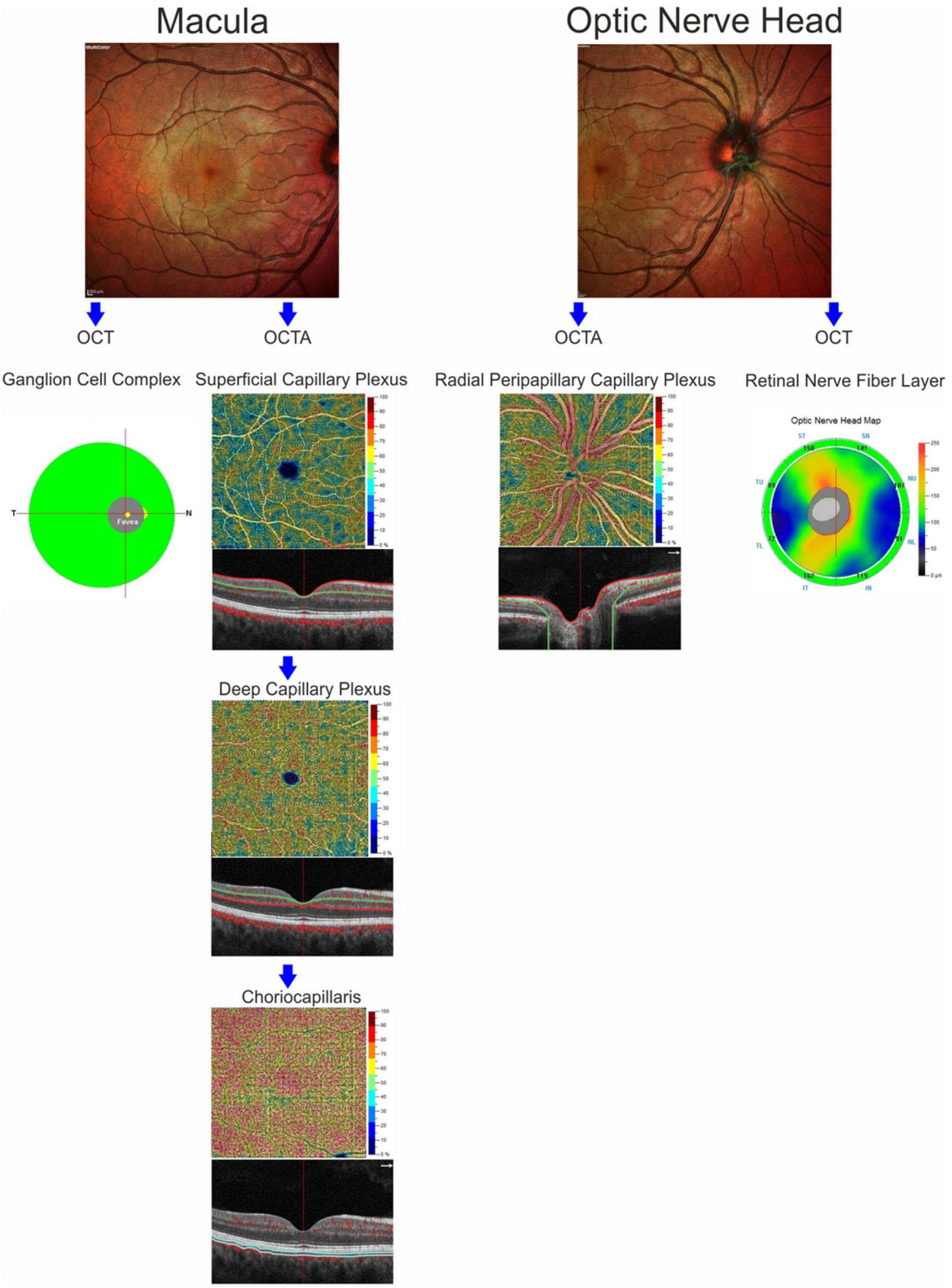

FIGURE 1 | Anatomic illustration of macular and optic nerve head regions showing the retinal structures analyzed by optical coherence tomography (ganglion cell complex and retinal nerve fiber layer) and the retinal and choriocapillaris vascular networks evaluated by OCT-Angiography.

TABLE 1 | Demographic and clinical characteristics of IDE, RRMS patients and healthy controls.

	Control	IDE	RR-MS
Eyes (N.)	30	40	20
Age, mean ± SD (years)	28.2 ± 8.6	28.9 ± 9.5	29.7 ± 6.3
Sex (female/male)	10/5	10/10	7/3
EDSS, mean ± SD (Range)	-	1.85 ± 0.95 (0–2.5)	2.3 ± 0.57 (1.5–3.5)
Annualized relapse rate, mean ± SD	-	-	0.99 ± 1.05
Disease duration, mean ± SD (years)	-	1.7 ± 2.3	4 ± 1.3
Onset modality			
Brainstem, N. (%)	-	6 (30%)	4 (40%)
Pyramidal, N. (%)	-	5 (25%)	2 (20%)
Cerebellar, N. (%)	-	1 (5%)	1 (10%)
Sensory, N. (%)	-	7 (35%)	3 (30%)
Bowel/Bladder, N. (%)	-	0 (0%)	0 (0%)
Cerebral, N. (%)	-	1 (5%)	0 (0%)
OCT-A parameters (%)			
Superficial capillary plexus, mean ± SD	53.63 ± 2.53	50.43 ± 4.47	48.75 ± 4.41
Deep capillary plexus, mean ± SD	55.97 ± 4.86	55.15 ± 6.53	53.25 ± 7.06
Choriocapillaris, mean ± SD	74.10 ± 2.66	74.08 ± 2.34	74.15 ± 2.54
Radial peripapillary plexus, mean ± SD	53.23 ± 3.35	49.62 ± 2.90	45.9 ± 3.93
OCT parameters (μm)			
Ganglion cell complex average, mean ± SD	100.2 ± 6.79	98.61 ± 9.89	89.54 ± 9.85
Retinal nerve fiber layer average, mean ± SD	103.1 ± 8.19	101.83 ± 10.88	95.15 ± 13.18
Visual Field parameters			
Mean Deviation, mean ± SD	−0.51 ± 1.18	−0.59 ± 1.61	−1.19 ± 2.09
Pattern standard deviation, mean ± SD	2.1 ± 0.46	2.42 ± 1.12	2.25 ± 0.83
Best-corrected visual acuity, mean ± SD (logMAR)	0.03 ± 0.04	0.02 ± 0.04	0.01 ± 0.03

IDE, Initial Demyelinating Event; RRMS, Relapsing–Remitting Multiple Sclerosis; EDSS, Expanded Disability Status Scale; OCT-A, Optical Coherence Tomography Angiography; logMAR, logarithm of the minimum angle of resolution; SD, Standard Deviation.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (Version 20.0 for Windows; SPSS Inc., Chicago, Ill, USA). One-way analysis of variance followed by Bonferroni *post hoc* analysis was used to evaluate differences in visual field parameters, age and best-corrected visual acuity among HCs, IDE and RR-MS patients. Chi-squared test was used to determine sex differences among groups. Linear mixed models, including subject, age and sex as covariates, was used to evaluate VD differences in each retinal vascular network (superficial capillary plexus, deep capillary plexus, radial peripapillary capillary plexus) and in choriocapillaris, using group as factor of interests. The same model was used to analyze differences in structural OCT parameters (ganglion cell complex average and retinal nerve fiber layer average) among the groups. Correlations between SD-OCT and OCTA parameters were assessed using linear mixed model for both IDE and RR-MS. Moreover, we analyzed the correlations between best-corrected visual acuity, mean deviation and pattern standard deviation, neurological (EDSS, annualized relapse rate, and disease duration) and OCT-A parameters. Since we evaluated VD in four different regions as dependent variables for the linear mixed models, to correct analysis for multiple regressions, we set the p-value for significance at $p = 0.05/4$ (0.012).

RESULTS

Demographic and Clinical Features

Thirty patients (20 with IDE and 10 with RR-MS) for a total of 60 eyes and 15 HCs for a total of 30 eyes, were enrolled. There were no significant differences for age, sex, best-corrected visual acuity, and visual field parameters in the three groups. After MRI evaluation, 16 out of 20 IDE (80%) patients met criteria for CIS whereas 4 IDE patients met MRI criteria for RR-MS. Demographic, clinical and OCT features are summarized in **Table 1**.

SD-OCT

At SD-OCT exam, RR-MS patients showed lower ganglion cell complex values compared with IDE patients (89.54 ± 9.85 vs. 98.61 ± 9.89; $p = 0.017$) and HCs (89.54 ± 9.85 vs. 100.2 ± 6.79; $p = 0.006$). Ganglion cell complex thickness was not different between IDE group and HCs. Retinal nerve fiber layer did not differ between HCs, IDE, and RR-MS patients.

OCT-A

The VD in radial peripapillary capillary plexus was significantly lower in IDE group compared with HCs (coeff. $\beta = -3.578$; $p = 0.002$). VD for both superficial capillary plexus and radial

TABLE 2 | Differences in OCTA parameters among IDE, RRMS and control groups.

OCT-A parameters	IDE vs. control		
	β	(95% CI)	*P*-value
Superficial capillary plexus	−3.180	(−5.696 to −0.664)	0.015
Deep capillary plexus	−0.534	(−4.021 to 2.952)	0.758
Choriocapillaris	−0.111	(−1.518 to 1.296)	0.874
Radial peripapillary capillary plexus	−3.578	(−5.724 to −1.431)	0.002
	RRMS vs. control		
	β	(95% CI)	*P*-value
Superficial capillary plexus	−4.955	(−7.933 to −1.977)	0.002
Deep capillary plexus	−2.996	(−7.122 to 1.131)	0.15
Choriocapillaris	0.129	(−1.536 to 1.794)	0.877
Radial peripapillary capillary plexus	−7.446	(−9.906 to −4.986)	<0.001
	RRMS vs. IDE		
	β	(95% CI)	*P*-value
Superficial capillary plexus	−1.775	(−4.361 to 1.080)	0.216
Deep capillary plexus	−2.461	(−6.418 to 1.496)	0.216
Choriocapillaris	0.240	(−1.357 to 1.837)	0.763
Radial peripapillary capillary plexus	−3.868	(−6.289 to −1.448)	0.003

IDE, Initial Demyelinating Event; RR-MS, Relapsing–Remitting Multiple Sclerosis; OCT-A, Optical Coherence Tomography Angiography; CI, Confidence Interval.

peripapillary capillary plexus was lower for RR-MS patients compared with HCs (coeff. $\beta = -4.955$; $p = 0.002$, and coeff. $\beta = -7.446$; $p < 0.001$, respectively; see **Table 2**). RR-MS patients showed a lower VD in radial peripapillary capillary plexus compared with IDE patients (coeff. $\beta = -3.868$; $p = 0.003$; see **Table 2**). The VD in choriocapillaris and deep capillary plexus did not differ between the three groups (see **Figure 2**). There were no significant correlations between OCT-A measures and visual field parameters (mean deviation and pattern standard deviation) while VD in the deep capillary plexus showed a significant correlation with best-corrected visual acuity (coeff. $\beta = -0.002$; $p = 0.007$; **Table 3**). No correlation was found between OCT-measures and neurological parameters (EDSS, annualized relapse-rate and disease duration).

OCT-A Correlates to SD-OCT

In patients, ganglion cell complex thickness was associated with VD in superficial capillary plexus and radial peripapillary capillary plexus (coeff. $\beta = 1.474$; $p < 0.001$ and coeff. $\beta = 1.101$; $p < 0.001$). Similarly, retinal nerve fiber layer thickness was associated with VD in radial peripapillary capillary plexus (coeff. $\beta = 0.817$; $p = 0.009$; **Table 4**).

DISCUSSION

Notwithstanding the many progresses achieved over the last years in uncovering different mechanisms contributing to tissue damage and clinical disability in MS, a full understanding of the disease pathogenesis is still hampered by the impossibility to study the premorbid stages of the disease. To overcome this obstacle, a valuable opportunity is provided by the exploration of pathology abnormalities occurring in very early disease phases. Specifically, we hereby investigated the role of vascular abnormalities in MS pathogenesis. We evaluated their role as early marker of disease, analyzing retinal and choriocapillaris VD in patients with recent onset of their first demyelinating episode. OCT-A is a reliable marker of disease and disability accrual in definite MS (8, 15, 18), especially in later stages. However, its role in earlier disease stages is less clear. To our knowledge, only two studies exploring VD variations enrolled early-stage MS patients, but in both cases these were considered in a pooled analysis including also RR-MS patients, making impossible to draw specific conclusions to early stage MS (9, 18). In the present study, the comparison of patients with IDE, RR-MS, and HCs in terms of retinal VD suggests an early involvement of the radial peripapillary capillary plexus, regardless of the presence of retinal atrophy or ongoing inflammation. Feucht and colleagues recently reported a reduced VD of the superficial capillary plexus and deep capillary plexus in eyes of MS and CIS patients affected by optic neuritis, with no changes in the healthy eye (9). In addition, Murphy et al. described a reduction of superficial capillary plexus in eyes affected by optic neuritis and, to a lesser extent, in the healthy eye (18). In our sample, we identified a rarefaction of radial peripapillary capillary plexus both in IDE and RRMS with no history of optic neuritis. Both previous studies (9, 18) described an association between inner retinal layer volumes and density of both the superficial and deep vascular plexuses, suggesting a relationship between retinal atrophy and the consequent reduction in vascularization, induced by the reduced metabolic request of the atrophic layers. In our study, similar associations were identified between OCT-A and structural-OCT parameters in the entire patients' group but, as no retinal atrophy was present in IDE patients, VD reduction in radial peripapillary capillary plexus should not be ascribed to macroscopic structural abnormalities of the retina nor to the presence of optic nerve atrophy. Radial peripapillary capillary plexus rarefaction could be indeed the proxy of a more diffuse vascular involvement in MS pathogenesis or, alternatively, it might be related to subtle microstructural changes of the optic nerve fibers, which might explain the selective VD reduction in radial peripapillary capillary plexus rather than in all the explored vascular districts. Fibers within the retinal nerve fiber layer might suffer indirectly from vascular damage of the optic nerve, in the frame of a more diffuse white matter microstructural damage, that has been described as an early finding in CIS patients (19, 20). As per the insight gained from RR-MS patients, later on in the disease course, radial peripapillary capillary plexus rarefaction increased, with superficial capillary plexus showing VD reduction too, mirroring the development of atrophy in retinal nerve fiber layer and ganglion cell complex. Finally, similarly to what reported by Feucht et al. for CIS/MS patients (9), abnormalities in choriocapillaris VD were not detected in our IDE/RRMS patients. Unfortunately, no formal analysis of the association between choriocapillaris VD and previous relapse rate could be

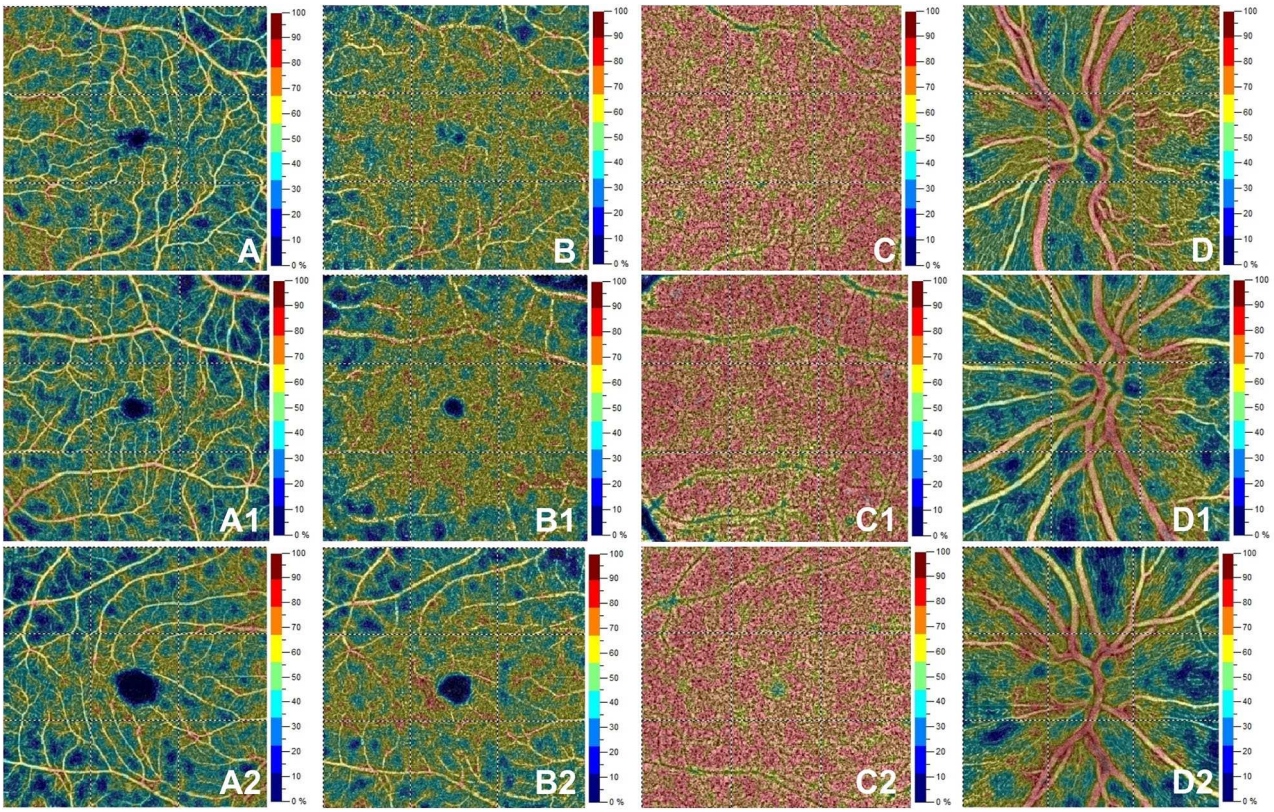

FIGURE 2 | Optical coherence tomography angiography (OCT-A) images from a healthy subject' s left eye (male, 28 years) in the first row show normal vessel density in superficial capillary plexus **(A)**, deep capillary plexus **(B)**, choriocapillaris **(C)** and radial peripapillary capillary plexus **(D)**. The second row depicts OCTA features in the left eye for a patient (female, 28 years) with initial demyelinating event. OCTA reveals normal vessel density in superficial capillary plexus **(A1)**, deep capillary plexus **(B1)**, choriocapillaris **(C1)** with a decrease for vessel density in the radial peripapillary capillary plexus **(D1)**. The bottom row shows a patient's right eye (male, 29 years) affected by relapsing-remitting multiple sclerosis. Here, vessel density is reduced in the superficial capillary plexus **(A2)** and radial peripapillary capillary plexus **(D2)** without vessel density changes in the deep capillary plexus **(B2)** and choriocapillaris **(C2)**.

performed, as the majority of our sample was constituted by IDE subjects for whom, by definition, no past disease activity is present in terms of more than one relapse. Furthermore, the lack of correlation between OCT-A and clinical parameters, which might seem counterintuitive, considering previous reports in MS (8, 15, 18), might be similarly accounted for by the mild clinical status of IDE patients. Eventually, due to the cross-sectional nature of the study, we cannot completely rule out that changes in VD without structural-OCT abnormalities might depend on the lower inter-subject variability of OCT-A measures compared with structural OCT measures. When analyzed over the follow-up, SD-OCT shows high level of sensibility for detecting retinal structural changes (21, 22). Longitudinal studies with a larger sample size are highly needed to evaluate the sensitivity for OCT-A in detecting progressive VD loss over the disease course regardless of the inter-subject variability and to assess the contribute of this technique to the already validated standard-OCT. In addition, it is worthy to mention that OCT-A might be more sensitive than SD-OCT in detecting retinal abnormalities associated with subclinical optic neuritis. When compared with visual evoked potentials, SD-OCT was shown to be less sensitive in detecting subclinical optic neuritis (23, 24). To explore this hypothesis a multimodal assessment of optic nerve through SD-OCT, OCT-A, visual evoked potentials and, eventually, MRI scans might shed further lights on this topic. In conclusion, our data suggest that retinal vascular abnormalities are possibly driven by primary vessel involvement, or secondary to structural damage ongoing in the retina and optic nerve during the disease course. The role played by each mechanism seems to differ according to the disease stage, with VD being the proxy of primary vessel involvement or subclinical white matter macrostructural abnormalities in an early stage, and retinal atrophy in a later stage. Regardless of the causative mechanism, our results confirm the relevant role of retinal VD as a non-invasive, early biomarker of disease, independently from the presence of inflammation, although we recognize that the applications of radial peripapillary capillary plexus VD measurements as diagnostic marker in clinical settings will require further studies to explore the specificity of such vessel density rarefaction.

TABLE 3 | Correlations between vessel density, visual field, and visual acuity for MS patients.

Regions	Mean deviation		
	β	(95% CI)	*P*-value
Superficial capillary plexus	0.079	(−0.023 to 0.182)	0.127
Deep capillary plexus	0.035	(−0.016 to 0.087)	0.173
Choriocapillaris	−0.046	(−0.189 to 0.097)	0.517
Radial peripapillary capillary plexus	−0.103	(−0.200 to −0.005)	0.038
	Pattern standard deviation		
	β	(95% CI)	*P*-value
Superficial capillary plexus	−0.018	(−0.098 to 0.062)	0.654
Deep capillary plexus	−0.015	(−0.058 to 0.028)	0.480
Choriocapillaris	−0.001	(−0.116 to 0.113)	0.978
Radial peripapillary capillary plexus	0.017	(−0.057 to 0.092)	0.637
	Best-corrected visual acuity		
	β	(95% CI)	*P*-value
Superficial capillary plexus	0.003	(−0.0001 to 0.006)	0.061
Deep capillary plexus	−0.002	(−0.005 to 0.0008)	0.007
Choriocapillaris	−0.0008	(−0.005 to 0.004)	0.733
Radial peripapillary capillary plexus	−0.0002	(−0.003 to 0.002)	0.855

CI, confidence interval.

TABLE 4 | Correlations between OCTA and OCT parameters in the group including IDE and RRMS patients.

OCT-A parameters	Ganglion cell complex average			Retinal nerve fiber layer average		
	β	(95% CI)	*P*-value	β	(95% CI)	*P*-value
Superficial capillary plexus	1.474	(0.910 to 2.039)	<0.001	0.486	(0.138 to 1.389)	0.123
Deep capillary plexus	−0.215	(−0.549 to 0.118)	0.197	−0.145	(−0.442 to 0.152)	0.321
Choriocapillaris	−0.166	(−0.983 to 0.649)	0.683	0.712	(−0.142 to 1.567)	0.098
Radial peripapillary plexus	1.101	(0.591 to 1.612)	<0.001	0.817	(0.218 to 1.416)	0.009

OCT-A, Optical Coherence Tomography Angiography; CI, Confidence Interval.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study, involving human participants, was reviewed and approved by the Institutional Review Board of the University of Naples Federico II and all investigations adhered to the tenets of the Declaration of Helsinki (protocol number: 142/19). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design of the study: GC, AC, RL, and VB. Data acquisition: RL, DM, AM, FT, GC, AS, MD, CC, MP, AL, MM, and MP. Data analysis: AC, DM, MP, and MM. Manuscript drafting: AC, GC, DM, MP, GS, VB, RL, and CC. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Petzold A, Balcer LJ, Calabresi PA, Costello F, Frohman TC, Frohman EM, et al. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol.* (2017) 16:797–812. doi: 10.1016/S1474-4422(17)30278-8
2. Costello F, Burton JM. Retinal imaging with optical coherence tomography: a biomarker in multiple sclerosis? *Eye Brain.* (2018) 10:47–63. doi: 10.2147/EB.S139417
3. Oertel FC, Zimmermann HG, Brandt AU, Paul F. Novel uses of retinal imaging with optical coherence tomography in multiple sclerosis. *Expert Rev Neurother.* (2019) 19:31–43. doi: 10.1080/14737175.2019.1559051

4. D'Haeseleer M, Hostenbach S, Peeters I, Sankari SE, Nagels G, De Keyser J, et al. Cerebral hypoperfusion: a new pathophysiologic concept in multiple sclerosis? *J Cereb Blood Flow Metab*. (2015) 35:1406–10. doi: 10.1038/jcbfm.2015.131
5. Moccia M, Lanzillo R, Palladino R, Maniscalco GT, De Rosa A, Russo C, et al. The Framingham cardiovascular risk score in multiple sclerosis. *Eur J Neurol*. (2015) 22:1176–83. doi: 10.1111/ene.12720
6. Wang X, Jia Y, Spain R, Potsaid B, Liu JJ, Baumann B, et al. Optical coherence tomography angiography of optic nerve head and parafovea in multiple sclerosis. *Br J Ophthalmol*. (2014) 98:1368–73. doi: 10.1136/bjophthalmol-2013-304547
7. Cennamo G, Romano MR, Vecchio EC, Minervino C, Della Guardia C, Velotti N, et al. Anatomical and functional retinal changes in multiple sclerosis. *Eye*. (2016) 30:456–62. doi: 10.1038/eye.2015.256
8. Lanzillo R, Cennamo G, Criscuolo C, Carotenuto A, Velotti N, Sparnelli F, et al. Optical coherence tomography angiography retinal vascular network assessment in multiple sclerosis. *Mult Scler*. (2018) 24:1706–14. doi: 10.1177/1352458517729463
9. Feucht N, Maier M, Lepennetier G, Pettenkofer M, Wetzlmair C, Daltrozzo T, et al. Optical coherence tomography angiography indicates associations of the retinal vascular network and disease activity in multiple sclerosis. *Mult Scler*. (2019) 25:224–34. doi: 10.1177/1352458517750009
10. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. (2018) 17:162–73. doi: 10.1016/S1474-4422(17)30470-2
11. Kniestedt C, Stamper RL. Visual acuity and its measurement. *Ophthalmol Clin North Am*. (2003) 170:155. doi: 10.1016/s0896-1549(03)00013-0
12. Cennamo G, Montorio D, Velotti N, Sparnelli F, Reibaldi M, Cennamo G. Optical coherence tomography angiography in pre-perimetric open-angle glaucoma. *Graefes Arch Clin Exp Ophthalmol*. (2017) 255:1787–93. doi: 10.1007/s00417-017-3709-7
13. Schippling S, Balk LJ, Costello F, Albrecht P, Balcer L, Calabresi PA, et al. Quality control for retinal OCT in multiple sclerosis: validation of the OSCAR-IB criteria. *Mult Scler*. (2015) 21:163–70. doi: 10.1177/1352458514538110
14. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, Saidha S, Martinez-Lapiscina EH, Lagreze WA, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology*. (2016) 86:2303–9. doi: 10.1212/WNL.0000000000002774
15. Lanzillo R, Cennamo G, Moccia M, Criscuolo C, Carotenuto A, Frattaruolo N, et al. Retinal vascular density in multiple sclerosis: a 1-year follow-up. *Eur J Neurol*. (2019) 26:198–201. doi: 10.1111/ene.13770
16. Huang D, Jia Y, Gao SS, Lumbroso B, Rispoli M. Optical coherence tomography angiography using the optovue device. *Dev Ophthalmol*. (2016) 56:6–12. doi: 10.1159/000442770
17. Rao HL, Pradhan ZS, Weinreb RN, Reddy HB, Riyazuddin M, Dasari S, et al. Regional comparisons of optical coherence tomography angiography vessel density in primary open-angle glaucoma. *Am J Ophthalmol*. (2016) 171:75–83. doi: 10.1016/j.ajo.2016.08.030
18. Murphy OC, Kwakyi O, Iftikhar M, Zafar S, Lambe J, Pellegrini N, et al. Alterations in the retinal vasculature occur in multiple sclerosis and exhibit 857 novel correlations with disability and visual function measures. *Mult Scler*. (2019). doi: 10.1177/1352458519845116. [Epub ahead of print].
19. Rocca MA, Preziosa P, Mesaros S, Pagani E, Dackovic J, Stosic-Opincal T, et al. Clinically isolated syndrome suggestive of multiple sclerosis: dynamic patterns of gray and white matter changes-A 2-year MR imaging study. *Radiology*. (2016) 278:841–53. doi: 10.1148/radiol.2015150532
20. Kugler AV, Deppe M. Non-lesional cerebellar damage in patients with clinically isolated syndrome: DTI measures predict early conversion into clinically definite multiple sclerosis. *Neuroimage Clin*. (2018) 19:633–9. doi: 10.1016/j.nicl.2018.04.028
21. Button J, Al-Louzi O, Lang A, Bhargava P, Newsome SD, Frohman T, et al. Disease-modifying therapies modulate retinal atrophy in multiple sclerosis: a retrospective study. *Neurology*. (2017) 88:525–32. doi: 10.1212/WNL.0000000000003582
22. Pisa M, Guerrieri S, Di Maggio G, Medaglini S, Moiola L, Martinelli V, et al. No evidence of disease activity is associated with reduced rate of axonal retinal atrophy in MS. *Neurology*. (2017) 89:2469–75. doi: 10.1212/WNL.0000000000004736
23. Naismith RT, Tutlam NT, Xu J, Klawiter EC, Shepherd J, Trinkaus K, et al. Optical coherence tomography differs in neuromyelitis optica compared with multiple sclerosis. *Neurology*. (2009) 72:1077–82. doi: 10.1212/01.wnl.0000345042.53843.d5
24. Di Maggio G, Santangelo R, Guerrieri S, Bianco M, Ferrari L, Medaglini S, et al. Optical coherence tomography and visual evoked potentials: which is more sensitive in multiple sclerosis? *Mult Scler*. (2014) 20:1342–7. doi: 10.1177/1352458514524293

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Evolution of Cortical and White Matter Lesion Load in Early-Stage Multiple Sclerosis: Correlation With Neuroaxonal Damage and Clinical Changes

*Ramona-Alexandra Todea[1,2†], Po-Jui Lu[1,3†], Mario Joao Fartaria[4,5,6], Guillaume Bonnier[1], Renaud Du Pasquier[7], Gunnar Krueger[8], Meritxell Bach Cuadra[5,9], Marios Nikos Psychogios[2], Ludwig Kappos[1,3], Jens Kuhle[3] and Cristina Granziera[1,3]**

[1] Translational Imaging in Neurology (ThINk) Basel, Department of Biomedical Engineering, Basel University Hospital, University of Basel, Basel, Switzerland, [2] Section of Neuroradiology, Department of Radiology, University Hospital of Basel, Basel, Switzerland, [3] Neurologic Clinic and Policlinic, Departments of Medicine, Clinical Research, and Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland, [4] Advanced Clinical Imaging Technology, Siemens Healthcare AG, Lausanne, Switzerland, [5] Department of Radiology, University Hospital and University of Lausanne, Lausanne, Switzerland, [6] Signal Processing Laboratory (LTS 5), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [7] Service of Neurology, Department of Clinical Neurosciences, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland, [8] Siemens Healthcare AG, Zurich, Switzerland, [9] Medical Image Analysis Laboratory (MIAL), Centre d'Imagerie BioMédicale (CIBM), Lausanne, Switzerland

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Paolo Preziosa, Vita-Salute San Raffaele University, Italy

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***Correspondence:**
Cristina Granziera
cristina.granziera@unibas.ch

[†] These authors have contributed equally to this work

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Introduction: Changes in cortical and white matter lesion (CL, WML) load are pivotal metrics to diagnose and monitor multiple sclerosis patients. Yet, the relationship between (i) changes in CL/WML load and disease progression and between (ii) changes in CL/WML load and neurodegeneration at early MS stages is not yet established. In this work, we have assessed the hypothesis that the combined CL and WML load as well as their 2-years evolution are surrogate markers of neurodegeneration and clinical progression at early MS stages. To achieve this goal, we have studied a group of RRMS patients and have investigated the impact of both CL and WML load on neuroaxonal damage as measured by serum neurofilament light chain (sNfL). Next, we have explored whether changes in CL/WML load over 2 years in the same cohort of early-MS are related to motor and cognitive changes.

Methods: Thirty-two RRMS patients (<5 years disease duration) underwent: (i) 3T MRI for CL/WML detection and clinical assessment at baseline and 2-years follow-up; and (ii) baseline blood test for sNfL. The correlation between the number and volume of CL/WML and sNfL was assessed by using the Spearman's rank correlation coefficient and a generalized linear model (GLM). A GLM was also used to assess the relationship between (i) the number/volume of new, enlarged, resolved, shrunken, stable lesions and (ii) the difference in clinical scores between two time-points.

Results: At baseline, sNfL levels correlated with both total CL count/volume ($\rho = 0.6/0.7$, Corr-$P < 0.017$/Corr-$P < 0.001$) and with total WML count/volume ($\rho = 0.6/0.6$, Corr-$P < 0.01$ for both). Baseline sNfL levels also correlated with new WML count/volume ($\rho = 0.6/0.5$, Corr-P < 0.01/Corr-P < 0.05) but not with new CL.

Longitudinal changes in CL and WML count and volume were significantly associated with (i) sustained attention, auditory information, processing speed and flexibility ($p < 0.01$), (ii) verbal memory ($p < 0.01$); (iii) verbal fluency ($p < 0.05$); and (iv) hand-motor function ($p < 0.05$).

Discussion : Changes in cortical and white matter focal damage in early MS patients correlate with global neuroaxonal damage and is associated to cognitive performances.

Keywords: early relapsing remitting multiple sclerosis, MRI, MP2RAGE, cortical lesions, serum neurofilamants

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system, which leads to the formation of focal demyelinating plaques in white and gray matter (1, 2). These lesions appear on a background of an inflammatory reaction—characterized by accumulation of lymphocytes and activated microglia—and show demyelination, in which axons are at least partially preserved (3). At all MS stages, white matter lesions (WML) are characterized by different levels of inflammatory activity, remyelination and axonal loss, with more evident ongoing activity in lesions of patients at early MS stages (4–7).

Cortical demyelination—which may be focal or diffuse—is also frequent in MS and present at early MS stages (8). Cortical lesions appear inflammatory and strongly associated with meningeal inflammation (8) and encompass plaques affecting both the cortex and the underlying white matter (leukocortical lesions), small perivascular lesions that completely located within cerebral cortex (intracortical lesions) and subpial cortical lesions (9, 10).

The presence and changes in cortical and white matter lesions (CL, WML) load are pivotal metrics for the management of multiple sclerosis (MS) patients (11).

The number of WML and CL in patients with suspicious symptoms of MS is a fundamental criterion for the diagnosis of the disease (12). WML number at baseline is predictive of conversion to MS at 20-years follow-up in patients with clinical isolated syndrome (13), and WML volume appears to be associated with disability, motor and cognitive outcome at long-term follow-up (14). The number of CL appears to correlate with disability and cognition in early MS stages and shows even stronger associations with those outcome measures than WML load (15). Besides, CL load is strongly and positively associated with cognitive dysfunction and with severe gray matter atrophy (10). Also, cortical pathology—better than WML load—is related to disability progression in all MS disease phenotypes (16) and extensive cortical damage at onset is associated with both florid inflammatory clinical activity and rapid occurrence of the progressive phase (16).

Regarding patient monitoring, the accumulation of focal damage (i.e., the increase in WML number) is one of the criteria that is currently used to follow-up therapy response and eventually therapy-switch in MS patients (17, 18).

Irreversible central nervous system damage occurs in the early phase of MS and significantly contributes to disability progression in later stages of the disease (19, 20). That is why it is currently accepted that early treatment favorably impacts the long-term outcomes of MS patients (17, 21, 22), reduces disability progression in patients with RRMS, and decreases the risk of developing clinically defined MS in patients with clinically isolated syndrome (23–26). Nonetheless, with the current plethora of MS therapies, it is of outmost importance to stratify patients that might benefit from more aggressive therapeutic regimens than others at early disease stages.

To date, it remains unclear (i) whether changes in CL/WML load during the first years of MS disease parallel changes in clinical outcome and (ii) whether CL/WML load in early MS is proportional to ongoing neurodegeneration.

In this work, we have assessed the hypothesis that—in early MS—the combined CL and WML load as well as the 2-years evolution of CL/WML number and volume are surrogate markers (i) of neurodegeneration and (ii) of clinical progression. To achieve this goal, we have studied a group of RRMS patients and have investigated the impact of both CL and WML load on neuroaxonal damage as measured by serum neurofilament light chain (sNfL) (27, 28). Next, we have explored whether changes in CL/WML load over 2 years in the same cohort of early-MS are related to motor and cognitive changes.

METHODS

Population and Clinical Assessment

We performed a retrospective analysis in a cohort of patients enrolled at Lausanne University Hospital. Thirty-two early RRMS patients with <5 years disease duration were enrolled in the study (TP1) and followed up 2 years later (TP2). Inclusion criteria for patients were the following: definite MS diagnosis according to the revised McDonald criteria 2017, <5 years disease duration at enrolment, age between 20 and 70 years old and no other neurological or psychiatric disorder more than 3 months after the last relapse and/or end of corticosteroid therapy. Exclusion criteria were: claustrophobia and contraindications to MRI.

Also, at both TP1 and TP2, each of the 32 subjects underwent advanced MRI and a clinical examination, and 25 of them had blood sampled to measure sNfL levels at TP1.

Clinical assessment was performed using: (i) Expanded Disability status scale (EDSS) (29), (ii) Multiple Sclerosis Functional Composite score (MSFC) (30), (iii) Brief Repeatable Battery of Neuropsychological Tests; (BRBN) (31), (iv) Hospital Anxiety and Depression scale (HAD) (32), (v) Fatigue Scale for

Motor and Cognitive functions (33). Physical disability of the patients was scored using the Expanded Disability Status Scale (EDSS). The difference between clinical scores at TP2 and TP1 (TP2–TP1) was used as a measure of clinical changes over time.

The institutional ethics review board approved the study and all patients gave their written informed consent.

MR Imaging Acquisition

Images were acquired on a 3T scanner (MAGNETOM Trio a Tim system, Siemens Healthcare, Erlangen, Germany) using a 32-channel head coil. The imaging protocol included: Magnetization-Prepared 2 Rapid Acquisitions Gradient Echo (MP2RAGE, TR/TI1/TI2 = 5,000/700/2,500 ms, vs = 1.0 × 1.0 × 1.2 mm^3, acquisition time: ∼8 min) (34) and 3D Fluid-attenuated inversion recovery (FLAIR, TR/TE/TI = 5,000/394/1,800 ms, vs = 1.0 × 1.0 × 1.2mm^3, acquisition time: ∼6 min).

Image Analysis

WML/CL were segmented by consensus by a neurologist and a neuroradiologist on 3D FLAIR and MP2RAGE images using ITK-SNAP [http://www.itksnap.org, (35)]. WML/CL number and volumes were then extracted from the segmented lesion masks using MATLAB.

The detection of CL and the definition of CL types was performed on MP2RAGE images, which are known to be more sensitive to cortical focal pathology than both MPRAGE and 3D FLAIR (36). Cortical lesions were segmented if they were characterized by a local cortical hypointensity on MP2RAGE compared to the surrounding gray matter and they had at least 1 mm in plane resolution and more than three pixels in size.

The experts who manually performed lesion detection were unaware of the patients clinical status and cognitive tests results.

MS lesions were then classified in five groups as proposed in (37) depending on their evolution between the two time-points: *new* (identifiable on the TP2 images but not on the TP1 images); *enlarged* (characterized by a diameter increased at TP2 by at least 50%); *resolved* (clearly visible on the TP1 images but not on the TP2 images); *shrunken* (characterized by a diameter decrease at TP2 by at least 50%); *stable*: do not follow any of the above criteria (**Figure 1**). For the segmentation of new, resolved, shrunken, enlarged and stable lesions, we applied an automated method developed in house (38).

Serum Neurofilaments Measuring

Serum neurofilament light chain levels were measured using an electrochemiluminescence-based immunoassay (27).

Statistical Analysis

Assessment of the relationship between (i) CL/WML load at baseline and baseline sNfL and (ii) 2-years changes in CL/WML load and baseline sNfL

In patients, we performed Spearman's correlations between baseline sNfL and baseline number/volume of CL/WML. We also performed Spearman's correlations between baseline sNfL and changes in number/volume of CL/WML at 2-years follow-up. *P*-values were obtained from the permutation test with a case resampling rate of 10,000. False discovery rate correction was performed by using the Benjamin-Hochberg procedure to account for multiple comparisons.

A univariate general linear model (GLM) was also performed to assess the relative contribution of CL and WML to sNfL variations, which were transformed by Box-Cox transformation to be normally distributed since the *p*-value of the Shapiro-Wilk test on the sNfL is <0.001. The best GLM model was selected by Akaike information criterion (AIC) to reduce the risks of overfitting and underfitting.

Assessment of the relationship between changes in CL/WML load and clinical changes

General linear model was performed using: (i) the number of new, enlarged, resolved, shrunken, stable lesions as well as the volume of new, enlarged, resolved, shrunken, stable lesions as predictors and (ii) the delta (TP2-TP1) of each cognitive, motor and disability score as outcome. We checked the delta of all measures for normality by the Shapiro-Wilk test and the following were Box-Cox transformed: PASAT, SRT-LTS, SRT-D, and SDMT. The delta of each measure to be transformed was rendered positive by subtracting the minimum of the delta and adding 0.01* the maximum of the delta to avoid having negative values in the Box-Cox transformation. Age, gender, number of education years, and the change of the anxiety and depression scores were considered as covariates. This cohort of stable patients did not exhibit any relapses between TP1 and TP2. Backward-stepwise analyses based on AIC were performed to select the best prediction model for each clinical score. Bonferroni correction was applied to correct for the familywise error rate. A leave-one-out cross-validation (LOOCV) was conducted to assess the prediction quality of each model measured by the Spearman's correlation coefficient between the true and predicted outcomes in the validation sets of all folds.

Statistical analysis was performed using the R-project for statistical computing (https://www.r-project.org/).

RESULTS

Our cohort of RRMS patients consisted of 32 subjects, 13 males, 19 females with age at enrollment 35 ± 9.9 years (mean ± standard deviation, range 20–70 years); follow-up interval 21.4 ± 2.5 months, (mean ± standard deviation, range 16–27 months). All patients were < 5 years from initial symptoms 32 ± 21.6 months (mean ± standard deviation, range 3–70 months) and disease diagnosis 26 ± 19.3 months (mean ± standard deviation, range 0–59 months) at TP1. 88% of patients were on treatment at the baseline and 94% on treatment at the follow-up.

At baseline, 76% of patients ($n = 24$) were on Interferon Beta, 15% ($n = 5$) on Fingolimod and 9% ($n = 3$) on Glatiramer acetate. Treated patients remained on the same treatment for the entire duration of the study. There was no corticosteroid therapy within the 3 months preceding the enrollment and follow-up MRI.

Clinical scores at the time of enrollment (TP1), at the follow-up (TP2) and the difference in clinical scores between the two time-points (TP2-TP1) as a measure of clinical changes over time are shown in **Table 1**.

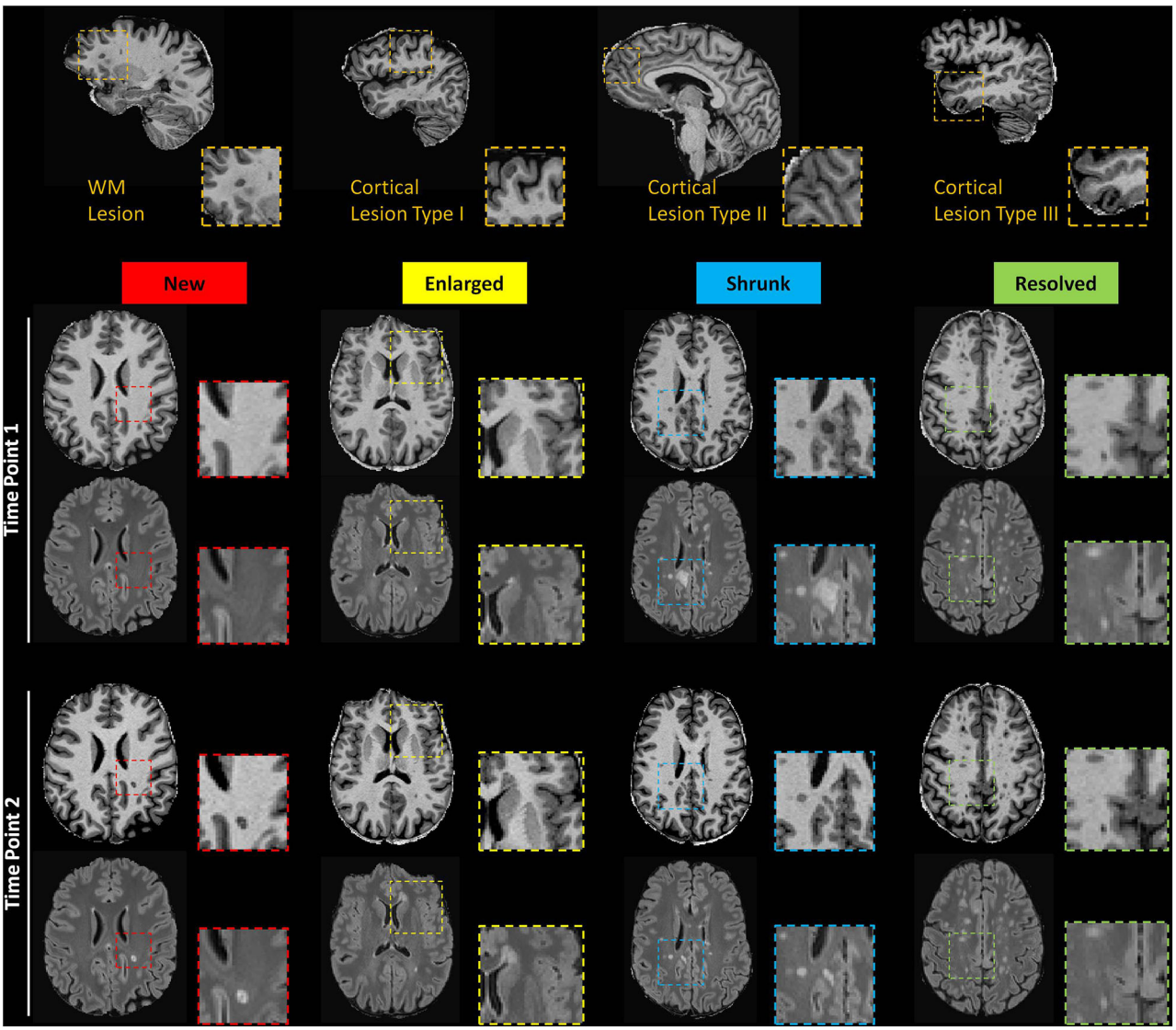

FIGURE 1 | Top row: Exemplary sagittal view in one patient showing WML and CL type 1, 2, and 3. Bottom rows: Axial slices of MP2RAGE and 3D FLAIR images showing exemplary new, enlarged, shrunken and resolved WML as automatically detected.

Longitudinal Changes in CL/WML

Baseline numbers and changes in WML and CL number over 2 years are reported in **Figures 2**, **3**.

Correlation Between sNfL and CL/WML at Baseline and With Changes in CL/WML Over 2 Years

At baseline, 164 (80.4%) of CL were type 1, 39 (19.1%) were type 2 and 1 only of type 3 (0.5%) in patients having measured sNfL. The sNfL levels in MS patients correlated with total CL count/volume ($\rho = 0.6/0.7$, Corr-P < 0.01/Corr-P < 0.001) to a similar extent than with total WML count/volume ($\rho = 0.6/0.6$, Corr-P < 0.01 for both), **Table 2**. Specifically, sNfL correlated with both CL-type I number/volume ($\rho = 0.5/0.6$, Corr-P < 0.05/Corr-P < 0.01) and with CL- type II number/volume ($\rho = 0.5/0.5$, Corr-P < 0.05 for both), **Table 2**.

The best GLM model included CL count/volume and WML volume as predictors and revealed a moderate association between sNfL at baseline and WML/CL volume (adj-$R^2 = 0.5$, $p = 0.0006$, pred-$R^2 = 0.09$). Besides, sNfL levels at baseline correlated with new WML count/volume ($\rho = 0.6/p = 0.5$, $p = 0.002/p = 0.01$, Corr-P < 0.01/Corr-P < 0.05) but not with new CL count/volume, **Table 2**.

Correlation Between Changes in CL/WML and Changes in Clinical Scores

Table 3 shows that the longitudinal changes in CL and WML volume and number were significantly associated with changes in:

TABLE 1 | Clinical scores at the time of enrollment (TP1), at the follow-up (TP2) and the difference in clinical scores between the two time-points (TP2-TP1) as a measure of clinical changes over time.

		TP1	TP2	TP2-TP1
Disability and motor function	EDSS	1.6 ± 0.3	1.7 ± 0.5	–
	9-HPT (Arm function)	19.8 ± 2.8	19.6 ± 2.7	−0.21 ± 2.13
	T25FWT (Leg function)	4 ± 0.8	3.4 ± 0.5	−0.62 ± 0.69
Cognition (BRB-N)	PASAT (cognitive)	46.8 ± 10.4	48.7 ± 11.1	1.84 ± 6.71
	SRT-LTS (verbal memory)	62.3 ± 7.2	65.7 ± 5	3.41 ± 6.32
	SRT-CLTR (verbal memory)	57.6 ± 11.4	61.6 ± 10	3.94 ± 9.15
	SRT-D (verbal memory)	11.4 ± 1.1	11.8 ± 0.5	0.38 ± 1.1
	SDMT (attention)	60.5 ± 17.3	57.2 ± 11.6	−3.34 ± 18.7
	SPART10/36 (visuospatial memory)	23.2 ± 4.3	23.2 ± 3.9	−0.03 ± 4.07
	WLG (verbal fluency)	27.6 ± 5.4	27.4 ± 7.9	−0.19 ± 5.4
Mood and fatigue	HAD-A (anxiety)	6 ± 4.1	5.7 ± 3.8	−0.28 ± 3.34
	HAD- D (depression)	2.9 ± 2.4	2.1 ±2.1	−0.78 ± 2.56
	FMSC-Cognitive	23 ± 8.4	22.7 ± 9.6	−0.31 ± 7.33
	FMSC-Motor	22.7 ± 9.6	23.1 ± 10.9	−0.84 ±7.41

Values are expressed in mean ± standard deviation unless otherwise indicated.
BRB-N, Brief repeatable battery of neuropsychological tests; EDSS, Expanded Disability Status Scale; T25FW, Timed 25-Foot Walk—leg function; 9-HPT, 9 Hole Peg Tests -arm function; BRB-N, Brief Repeatable Battery of Neuropsychological Tests; PASAT, paced auditory serial addition test; SRT-LTS, selective reminding test-long term storage; SRT-CLTR, selective reminding test—Consistent long-term storage; SRT-D, selective reminding test-delayed; SDMT, symbol digit modalities test; cSPART 10/36, Spatial Recall Test; WLG: word list generation; HAD-A, Hospital Anxiety and Depression scale- Anxiety; HAD-D, Hospital Anxiety and Depression scale-Depression; FMSC, Fatigue scale for Motor and Cognitive functions.

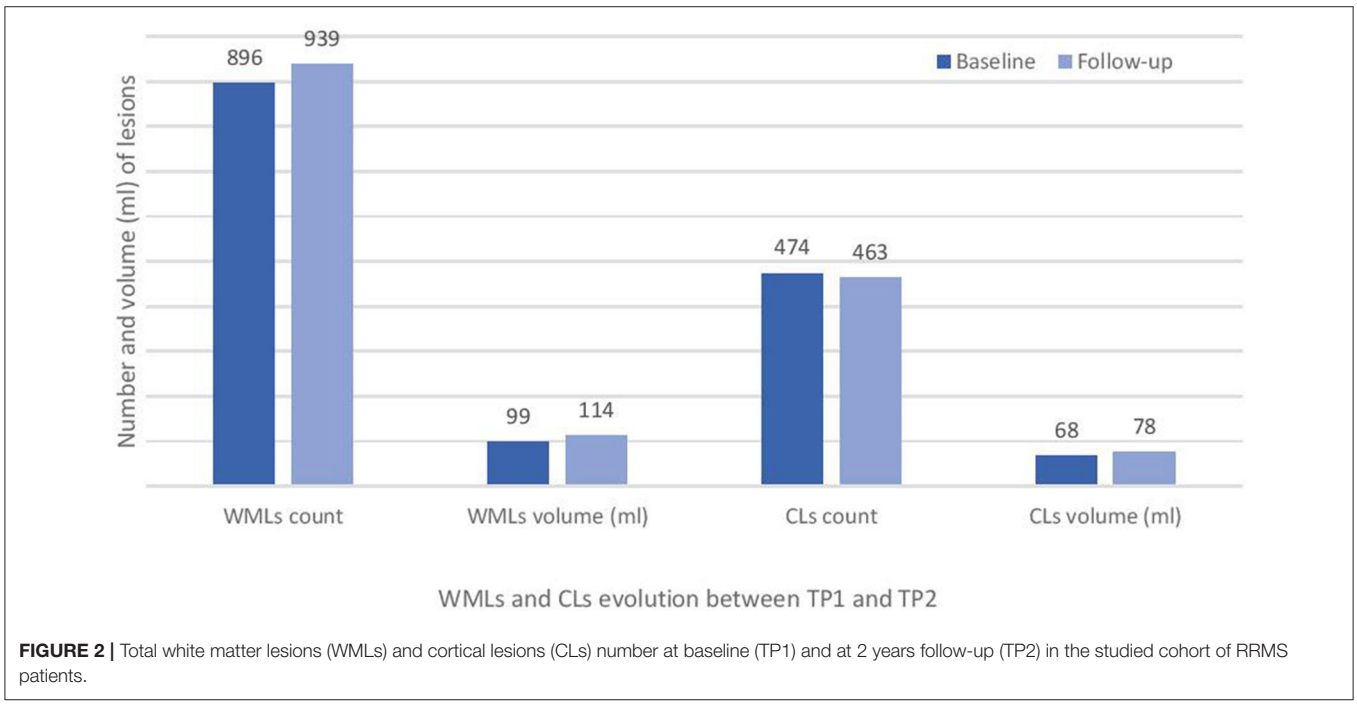

FIGURE 2 | Total white matter lesions (WMLs) and cortical lesions (CLs) number at baseline (TP1) and at 2 years follow-up (TP2) in the studied cohort of RRMS patients.

(i) Hand function (9HPT, adj-R^2: 0.5, Corr-$P = 0.03$, and $\rho = 0.5$ after leave-one-out cross-validation, LOOCV)
(ii) Sustained attention, auditory information, processing speed and flexibility (PASAT, adj-R^2: 0.5, Corr-$P = 0.01$, and $\rho = 0.5$ after leave-one-out cross-validation, LOOCV)
(iii) Verbal memory (SRT-D, adj-R^2: 0.5, $P = 0.01$ and $\rho = 0.45$ after LOOCV)
(iv) Semantic verbal fluency (WLG- word list generation test, adj-R^2: 0.5, Corr-$P = 0.05$, and $\rho = 0.4$ after LOOCV)

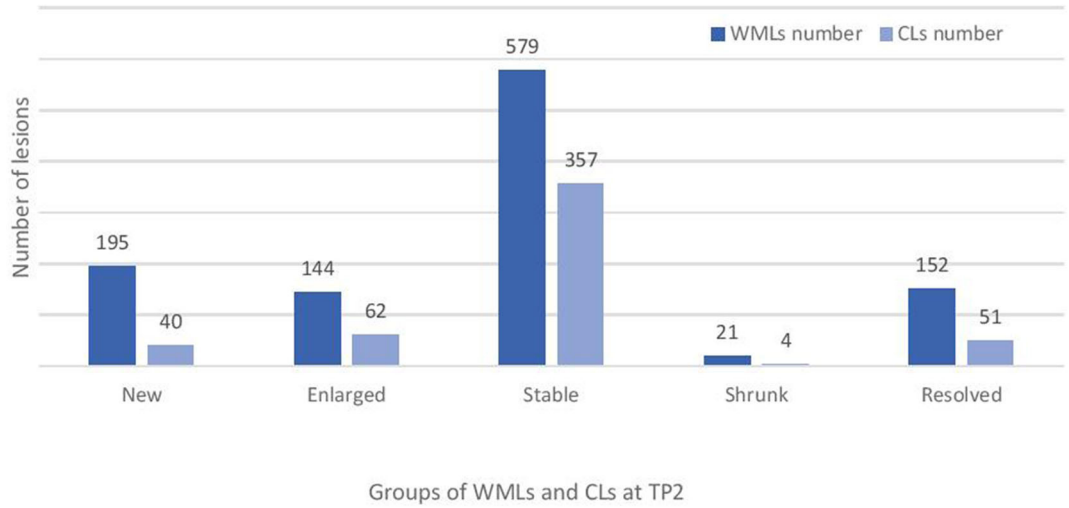

FIGURE 3 | Total number of new, enlarged, stable and resolved cortical and white matter lesions at follow-up (TP2) in the studied cohort of RRMS patients. WMLs, white matter lesions; CLs, cortical lesions.

TABLE 2 | Correlation between baseline sNfL with baseline WML/CL and new WML/CL load.

	Number			Volume		
	Spearman ρ	***P*-value**	**Corr-P**	**Spearman ρ**	***P*-value**	**Corr-P**
WML	0.58	0.003	**0.003**	0.61	0.002	**0.003**
CL	0.60	0.002	**0.003**	0.69	0.0002	**0.0006**
Lesion Type 1	0.54	0.005	**0.01**	0.64	0.0008	**0.003**
Lesion Type 2	0.45	0.025	**0.025**	0.50	0.013	**0.018**
New WML	0.58	0.002	**0.009**	0.51	0.01	**0.022**
New CL	0.38	0.06	0.085	0.31	0.13	0.13

P-values and Corr-P given are before and after Benjamini-Hochberg procedure, respectively. Bold values indicates the statistically significant values.

Specifically, changes in 9-HPT scores were associated with the gender ($p < 0.05$), number ($p < 0.05$) and volume ($p < 0.01$) of new lesions, number of enlarged lesions ($p < 0.05$) and number of shrunken lesions ($p < 0.05$), **Table 1 Supplementary data**.

Changes in PASAT (sustained attention, auditory information, processing speed, and flexibility) score were significantly associated with the patients age ($p < 0.01$) and number of CL/WML that shrunk in size ($p < 0.05$), **Table 2 Supplementary data**.

Changes in SRT-D were mainly associated with resolved CL/WML volume ($p < 0.001$), stable CL/WML volume ($p < 0.001$), new CL/WML number ($p < 0.05$), resolved CL/WML number ($p < 0.001$), sex ($p < 0.01$), **Table 3 Supplementary data**.

Changes in WLG test (semantic verbal fluency) was associated to the shrunken CL/WML volume ($p < 0.01$), stable CL/WML volume ($p < 0.001$), shrunken CL/WML number ($p < 0.05$), stable CL/WML number ($p < 0.01$), Hospital Anxiety and Depression scale-Depression ($p < 0.05$), **Table 4 Supplementary data**.

DISCUSSION

Our work shows that the number and volume of focal CL and WML are moderately related to neuroaxonal damage—as measured by sNfL—at early MS stages. We also determined that the changes in CL/WML load are associated with changes in cognition and in motor performance in our cohort of patients with short disease duration and on stable therapy.

MS is characterized by multifocal inflammatory processes, which lead to the formation of demyelinating lesions in cortical gray and white matter. These inflammatory processes dominate in early stages of the disease and can be targeted by current anti-inflammatory treatments (39), thereby slowing the accumulation of disability (40). Hence, early biomarkers of ongoing disease activity are fundamental to judge on the need of therapy-switch and escalation at early disease stages (41).

In this work, we have studied patients with early RRMS and mild physical disability, who were on first-line treatment at time of enrollment.

We assessed whether CL and WML load and their changes over 2 years might be a useful biomarker to quantify neuroaxonal damage in those patients. To assess neurodegeneration, we used a serum biomarker i.e., sNfL, since a previous study in the same cohort showed the absence of brain atrophy over the 2-years follow-up (42).

We found a moderate correlation between CL and WML load at baseline and sNfL measures at the same time point, confirming and extending previous knowledge that focal WM lesions affect overall neuroaxonal damage in patients with MS (43, 44). The measure of sNfL levels at baseline also showed a correlation with the increase in WML number over 2 years. These findings confirm and extend previous knowledge that sNfL levels are related to WML volume at 2 years follow-up in MS

TABLE 3 | Multiple regression between change of MRI metrics and change of clinical scores.

	Stepwise regression				LOOCV		
	Adjusted-R^2	*P*-value	Minimum, maximum, lambda	Corr-P	Spearman ρ	*P*-value	Corr-P
T25FWT	0.07	0.233	—	1	—	—	—
9-HPT	0.48	0.003	—	**0.03**	0.52	0.002	**0.02**
PASAT	0.46	0.001	(−11,24,0.4)	**0.01**	0.56	0.001	**0.008**
SRT-LTS	0.39	0.004	(−8,21,0.4)	**0.04**	0.4	0.025	0.2
SRT-CLTR	0.24	0.03	—	0.27	0.43	0.013	0.1
SRT-D	0.57	0.0003	(−2,4,0.8)	**0.003**	0.45	0.009	0.08
SPART 10/36	0.07	0.074	—	0.66	—	—	—
WLG	0.43	0.003	—	**0.03**	0.64	0.0001	**0.001**
SDMT	0.04	0.146	(−96,16,2.4)	1	—	—	—

Stepwise regression: The given P-values and Corrected P-values (Corr-P) are before and after Bonferroni correction, respectively. LOOCV: Spearman's rank correlation coefficient (ρ) between real and predicted outcome obtained through "leave-one-out" cross-validation (LOOCV). T25FWT, Timed25-Foot Walk Test- leg function; 9-HPT, 9-Hole Peg Test—arm function; PASAT, paced auditory serial addition test; SRT-LTS, selective reminding test-long-term storage; SRT-CLTR, selective reminding test-consistent long-term storage; SRT-D, selective reminding test-delayed recall; SPART 10/36, spatial recall test; WLG, word list generation; SDMT, symbol digit modality test. Bold values indicates the statistically significant values.

patient at more advanced disease stage (43); additionally, these data suggest that sNfL measurements at baseline may provide important complementary information over WM disease activity during the 2 years that follow but not of CL activity.

Interestingly, we did not measure any significant correlation between sNfL and changes in CL at 2 years follow-up, which is probably due to the low number of CL compared to WML in our cohort of patients.

We also showed that changes in size and number of lesions were strongly associated with changes in cognition (sustained attention, processing speed and flexibility as well as in spatial memory and semantic verbal fluency) but also with changes in hand motor function. It is known that some lesions—especially the recent ones—may shrink in size over time and their intensity on T2-weighted (i.e., FLAIR) images decreases as edema resolves and some tissue repair occurs, leaving a smaller lesion or an undetectable plaque (45). Other lesions undergo little changes in size (stable lesions) and some others significantly increase in volume over-time (e.g., lesions with chronic activity) (46). Much is known about the relationship between new and enlarging lesions and clinical outcome in MS (47, 48) but there is currently little knowledge about the contribution of shrinking and resolving lesions. Our results provide a new window into the complex changes in CL and WML, which influence mild changes in cognition and motor function in early MS patients on therapy.

Remarkably, our data also provide evidence that the reparatory activity in focal plaques– as measured through the number and volume of resolved and shrunk lesions—appear to strongly correlate with cognitive changes in our cohort of patients. Since a comprehensive cognitive assessment in clinical practice may be time consuming and unrealistic for routine follow-up of MS patients, the detection of new CL and WML during the early stage of the disease may support with alternative monitoring tools.

Detection of CL and of changes in WML and CL load in clinical practice is challenging. We have assessed the number and volume of cortical lesions and their changes over time by using MP2RAGE; this is a clinically available MR sequence that has shown similar sensitivity to double inversion recovery (DIR) for CL detection (36) and that appears to be artifact free in contrast to DIR (49). MP2RAGE may therefore provide the opportunity—together with a 3D FLAIR sequence for optimal WML detection–to assess the overall burden of focal activity in early MS patients in clinical practice.

Limitations of this study are the relatively small and homogeneous sample size and the fact that, due to the moderate number of patients studied, we could not consider treatment as a covariate in our regression models. We also acknowledge that the absence of information about gadolinium-enhancement at the time of the MRI might have influenced the sNFL results although this is not highly probable since patients were clinically stable and on therapy. In addition, we did not have a matched population of healthy controls to determine whether the measured sNFL levels were increased in patients. Future work should confirm these finding in larger cohorts of patients, including subjects with higher disability scores and disease activity as well as healthy controls.

In summary, our results suggest that early assessment of CL/WML load and their short-term evolution during the first year of disease are sensitive to ongoing axonal damage and related to subtle clinical changes. New efforts should be devoted to using these metrics to stratify patients at the beginning of the disease and hence to identify the ones who need more aggressive first-line therapies or therapeutic escalation.

DATA AVAILABILITY STATEMENT

Images and detailed clinical scores may be available upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethic committee of Lausanne University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CG: conceptualization, funding acquisition, and supervision. CG, P-JL, MF, R-AT, and GK: methodology. CG, P-JL, MF, and R-AT: formal analysis and investigation. R-AT, P-JL, and CG: writing—original draft preparation. R-AT, CG, MF, GB, RD, GK, MB, MP, LK, and JK: writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00973/full#supplementary-material

REFERENCES

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med.* (2000) 343:938–52. doi: 10.1056/NEJM200009283431307
2. Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain Pathol.* (1996) 6:259–74. doi: 10.1111/j.1750-3639.1996.tb00854.x
3. Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. *Brain Pathol.* (2007) 17:210–8. doi: 10.1111/j.1750-3639.2007.00064.x
4. Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Bruck W. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain.* (2002) 125(Pt 10):2202–12. doi: 10.1093/brain/awf235
5. Schultz V, van der Meer F, Wrzos C, Scheidt U, Bahn E, Stadelmann C, et al. Acutely damaged axons are remyelinated in multiple sclerosis and experimental models of demyelination. *Glia.* (2017) 65:1350–60. doi: 10.1002/glia.23167
6. Goldschmidt T, Antel J, Konig FB, Bruck W, Kuhlmann T. Remyelination capacity of the MS brain decreases with disease chronicity. *Neurology.* (2009) 72:1914–21. doi: 10.1212/WNL.0b013e3181a8260a
7. Ciccarelli O, Barkhof F, Bodini B, De Stefano N, Golay X, Nicolay K, et al. Pathogenesis of multiple sclerosis: insights from molecular and metabolic imaging. *Lancet Neurol.* (2014) 13:807–22. doi: 10.1016/S1474-4422(14)70101-2
8. Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, et al. Inflammatory cortical demyelination in early multiple sclerosis. *N Engl J Med.* (2011) 365:2188–97. doi: 10.1056/NEJMoa1100648
9. Bo L, Geurts JJ, Mork SJ, van der Valk P. Grey matter pathology in multiple sclerosis. *Acta Neurol Scand Suppl.* (2006) 183:48–50. doi: 10.1111/j.1600-0404.2006.00615.x
10. Calabrese M, Poretto V, Favaretto A, Alessio S, Bernardi V, Romualdi C, et al. Cortical lesion load associates with progression of disability in multiple sclerosis. *Brain.* (2012) 135(Pt 10):2952–61. doi: 10.1093/brain/aws246
11. Castro-Borrero W, Graves D, Frohman TC, Flores AB, Hardeman P, Logan D, et al. Current and emerging therapies in multiple sclerosis: a systematic review. *Ther Adv Neurol Disord.* (2012) 5:205–20. doi: 10.1177/1756285612450936
12. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology.* (2014) 83:278–86. doi: 10.1212/WNL.0000000000000560
13. Fisniku LK, Brex PA, Altmann DR, Miszkiel KA, Benton CE, Lanyon R, et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain.* (2008) 131(Pt 3):808–17. doi: 10.1093/brain/awm329
14. Rudick RA, Lee JC, Simon J, Fisher E. Significance of T2 lesions in multiple sclerosis: a 13-year longitudinal study. *Ann Neurol.* (2006) 60:236–42. doi: 10.1002/ana.20883
15. Rinaldi F, Calabrese M, Grossi P, Puthenparampil M, Perini P, Gallo P. Cortical lesions and cognitive impairment in multiple sclerosis. *Neurol Sci.* (2010) 31 (Suppl. 2):S235–7. doi: 10.1007/s10072-010-0368-4
16. Scalfari A, Neuhaus A, Degenhardt A, Rice GP, Muraro PA, Daumer M, et al. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. *Brain.* (2010) 133(Pt 7):1914–29. doi: 10.1093/brain/awq118
17. Río J, Castilló J, Rovira A, Tintoré M, Sastre-Garriga J, Horga A, et al. Measures in the first year of therapy predict the response to interferon beta in MS. *Mult Scler.* (2009) 15:848–53. doi: 10.1177/1352458509104591
18. Sormani MP, Rio J, Tintorè M, Signori A, Li D, Cornelisse P, Stubinski B, Stromillo M, Montalban X, De Stefano. N., Scoring treatment response in patients with relapsing multiple sclerosis. *Mult Scler.* (2013) 19:605–12. doi: 10.1177/1352458512460605
19. Freedman MS. Multiple sclerosis therapeutic strategies: use second-line agents as first-line agents when time is of the essence. *Neurol Clin Pract.* (2011) 1:66–8. doi: 10.1212/CPJ.0b013e31823cc2c2
20. Gold R, Wolinsky JS, Amato MP, Comi G. Evolving expectations around early management of multiple sclerosis. *Ther Adv Neurol Disord.* (2010) 3:351–67. doi: 10.1177/1756285610385608
21. Bermel RA, You X, Foulds P, Hyde R, Simon JH, Fisher E, et al. Predictors of long-term outcome in multiple sclerosis patients treated with interferon β. *Ann Neurol.* (2013) 73:95–103. doi: 10.1002/ana.23758
22. Giovannoni G, Butzkueven H, Dhib-Jalbut S, Hobart J, Kobelt G, Pepper G, et al. Brain health: time matters in multiple sclerosis. *Mult Scler Relat Disord.* (2016) 9 (Suppl. 1):S5–S48. doi: 10.1016/j.msard.2016.07.003
23. Jacobs LD, Beck RW, Simon JH, Kinkel RP, Brownscheidle CM, Murray TJ, et al. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. *CHAMPS Study Group. N Engl J Med.* (2000) 343:898–904. doi: 10.1056/NEJM200009283431301
24. Comi G, Filippi M, Barkhof F, Durelli L, Edan G, Fernández O, et al.T.o.M.Group SS. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. *Lancet.* (2001) 357:1576–82. doi: 10.1016/S0140-6736(00)04725-5
25. Comi G, Martinelli V, Rodegher M, Moiola L, Bajenaru O, Carra A, et al. Effect of glatiramer acetate on conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome (PreCISe study): a randomised, double-blind, placebo-controlled trial. *Lancet.* (2009). 374:1503–11. doi: 10.1016/S0140-6736(09)61259-9
26. Kappos L, Traboulsee A, Constantinescu C, Erälinna JP, Forrestal F, Jongen P, et al. Long-term subcutaneous interferon beta-1a therapy in patients with relapsing-remitting MS. *Neurology.* (2006) 67:944–53. doi: 10.1212/01.wnl.0000237994.95410.ce

27. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al.M.S.Group CS. Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. *Ann Neurol.* (2017) 81:857–70. doi: 10.1002/ana.24954
28. Siller N, Kuhle J, Muthuraman M, Barro C, Uphaus T, Groppa S, et al. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. *Mult Scler.* (2019) 25:678–86. doi: 10.1177/1352458518765666
29. Kurtzke JF, Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* (1983) 33:1444–52. doi: 10.1212/WNL.33.11.1444
30. Fischer JS, Rudick RA, Cutter GR, Reingold SC. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. *Mult Scler.* (1999) 5:244–50. doi: 10.1177/135245859900500409
31. Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. Frequency I, patterns, and prediction. *Neurology.* (1991) 41:685–91. doi: 10.1212/WNL.41.5.685
32. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand.* (1983) 67:361–70. doi: 10.1111/j.1600-0447.1983.tb09716.x
33. Penner IK, Raselli C, Stöcklin M, Opwis K, Kappos L, Calabrese P. The Fatigue Scale for Motor and Cognitive Functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler.* (2009) 15:1509–17. doi: 10.1177/1352458509348519
34. Marques JP, Kober T, Krueger G, van der Zwaag W, Van de Moortele PF, Gruetter R. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. *Neuroimage.* (2010) 49:1271–81. doi: 10.1016/j.neuroimage.2009.10.002
35. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage.* (2006) 31:1116–28. doi: 10.1016/j.neuroimage.2006.01.015
36. Kober T, Granziera C, Ribes D, Browaeys P, Schluep M, Meuli R, et al. MP2RAGE multiple sclerosis magnetic resonance imaging at 3T. *Invest Radiol.* (2012) 47:346–52. doi: 10.1097/RLI.0b013e31824600e9
37. Moraal B, Wattjes MP, Geurts JJ, Knol DL, van Schijndel RA, Pouwels PJ, et al. Improved detection of active multiple sclerosis lesions: 3D subtraction imaging. *Radiology.* (2010) 255:154–63. doi: 10.1148/radiol.09090814
38. Fartaria MJ, Kober T, Granziera C, Bach Cuadra. M., Longitudinal analysis of white matter and cortical lesions in multiple sclerosis. *Neuroimage Clin.* (2019) 23:101938. doi: 10.1016/j.nicl.2019.101938
39. Lassmann H, Targets of therapy in progressive MS. *Mult Scler.* (2017) 23:1593–9. doi: 10.1177/1352458517729455
40. Ziemssen T, De Stefano N, Sormani MP, Van Wijmeersch B, Wiendl H, Kieseier BC. Optimizing therapy early in multiple sclerosis: an evidence-based view. *Mult Scler Relat Disord.* (2015) 4:460–9. doi: 10.1016/j.msard.2015.07.007
41. Gasperini C, Prosperini L, Tintoré M, Sormani MP, Filippi M, Rio J, et al. Unraveling treatment response in multiple sclerosis: a clinical and MRI challenge. *Neurology.* (2019). 92:180–92. doi: 10.1212/WNL.0000000000006810
42. Bonnier G, Marechal B, Fartaria MJ, Falkowskiy P, Marques JP, Simioni S, et al. The combined quantification and interpretation of multiple quantitative magnetic resonance imaging metrics enlightens longitudinal changes compatible with brain repair in relapsing-remitting multiple sclerosis patients. *Front Neurol.* (2017) 8:506. doi: 10.3389/fneur.2017.00506
43. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Y, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. *Brain.* (2018) 141:2382–91. doi: 10.1093/brain/awy154
44. Magliozzi R, Howell OW, Nicholas R, Cruciani C, Castellaro M, Romualdi C, et al. Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. *Ann Neurol.* (2018) 83:739–55. doi: 10.1002/ana.25197
45. Rovira A, Auger C, Alonso J. Magnetic resonance monitoring of lesion evolution in multiple sclerosis. *Ther Adv Neurol Disord.* (2013) 6:298–310. doi: 10.1177/1756285613484079
46. Dal-Bianco A, Grabner G, Kronnerwetter C, Weber M, Hoftberger R, Berger T, et al. Slow expansion of multiple sclerosis iron rim lesions: pathology and 7 T magnetic resonance imaging. *Acta Neuropathol.* (2017) 133:25–42. doi: 10.1007/s00401-016-1636-z
47. Lavery AM, Verhey LH, Waldman AT. Outcome measures in relapsing-remitting multiple sclerosis: capturing disability and disease progression in clinical trials. *Mult Scler Int.* (2014) 2014:262350. doi: 10.1155/2014/262350
48. Sormani MP, Bruzzi P. MRI lesions as a surrogate for relapses in multiple sclerosis: a meta-analysis of randomised trials. *Lancet Neurol.* (2013) 12:669–76. doi: 10.1016/S1474-4422(13)70103-0
49. Geurts JJ, Barkhof F. Grey matter pathology in multiple sclerosis. *Lancet Neurol.* (2008) 7:841–51. doi: 10.1016/S1474-4422(08)70191-1

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PERSPECTIVE
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Revisiting the Pathoetiology of Multiple Sclerosis: Has the Tail Been Wagging the Mouse?

Monokesh K. Sen[1], *Mohammed S. M. Almuslehi*[1,2], *Peter J. Shortland*[3], *Jens R. Coorssen*[4] *and David A. Mahns*[1]*

[1] School of Medicine, Western Sydney University, Penrith, NSW, Australia, [2] Department of Physiology, College of Veterinary Medicine, University of Diyala, Baqubah, Iraq, [3] School of Science, Western Sydney University, Penrith, NSW, Australia, [4] Departments of Health Sciences and Biological Sciences, Faculties of Applied Health Sciences and Mathematics & Science, Brock University, St. Catharines, ON, Canada

Multiple Sclerosis (MS) is traditionally considered an autoimmune-mediated demyelinating disease, the pathoetiology of which is unknown. However, the key question remains whether autoimmunity is the initiator of the disease (outside-in) or the consequence of a slow and as yet uncharacterized cytodegeneration (oligodendrocytosis), which leads to a subsequent immune response (inside-out). Experimental autoimmune encephalomyelitis has been used to model the later stages of MS during which the autoimmune involvement predominates. In contrast, the cuprizone (CPZ) model is used to model early stages of the disease during which oligodendrocytosis and demyelination predominate and are hypothesized to precede subsequent immune involvement in MS. Recent studies combining a boost, or protection, to the immune system with disruption of the blood brain barrier have shown CPZ-induced oligodendrocytosis with a subsequent immune response. In this Perspective, we review these recent advances and discuss the likelihood of an inside-out vs. an outside-in pathoetiology of MS.

Keywords: inside-out, outside-in, immune response, oligodendrocytosis, cuprizone, experimental autoimmune encephalomyelitis, demyelination, CNS disorder

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***Correspondence:**
David A. Mahns
d.mahns@westernsydney.edu.au

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INTRODUCTION

Sir Robert Carswell, in his account of spinal cord lesions in humans, was the first to described demyelination in the human central nervous system (CNS) in 1838. These lesions were accompanied by atrophy and discolouration that were termed "*a peculiar disease*," (1) but made no attribution to Multiple Sclerosis (MS). Twenty-eight years later, in 1866, the French neurologist Jean-Martin Charcot first described these lesions as MS, "*la Sclerose en plaques disseminee*," and delineated it from other neurological diseases such as neurosyphilis, epilepsy, and progressive amyotrophies. Charcot also established diagnostic criteria based on the loss of myelin, thickening of small blood vessels, and the presence of fatty macrophages around vessels. In addition, he described that axons were more resistant to injury than myelin and the disability was linked to the axonal damage [reviewed by (2)]. Over 150 years (1866–2020) from the initial description, demyelination in the CNS remains the central McDonald criterion for MS in current diagnostic schemes (i.e., two or more separate episodes of hyperintense demyelinating lesions in at least two or more separate CNS locations) (3). Although the presence of oligoclonal bands in the cerebrospinal fluid of MS patients is used as an additional diagnostic criterion (3), the antigen(s) against which these

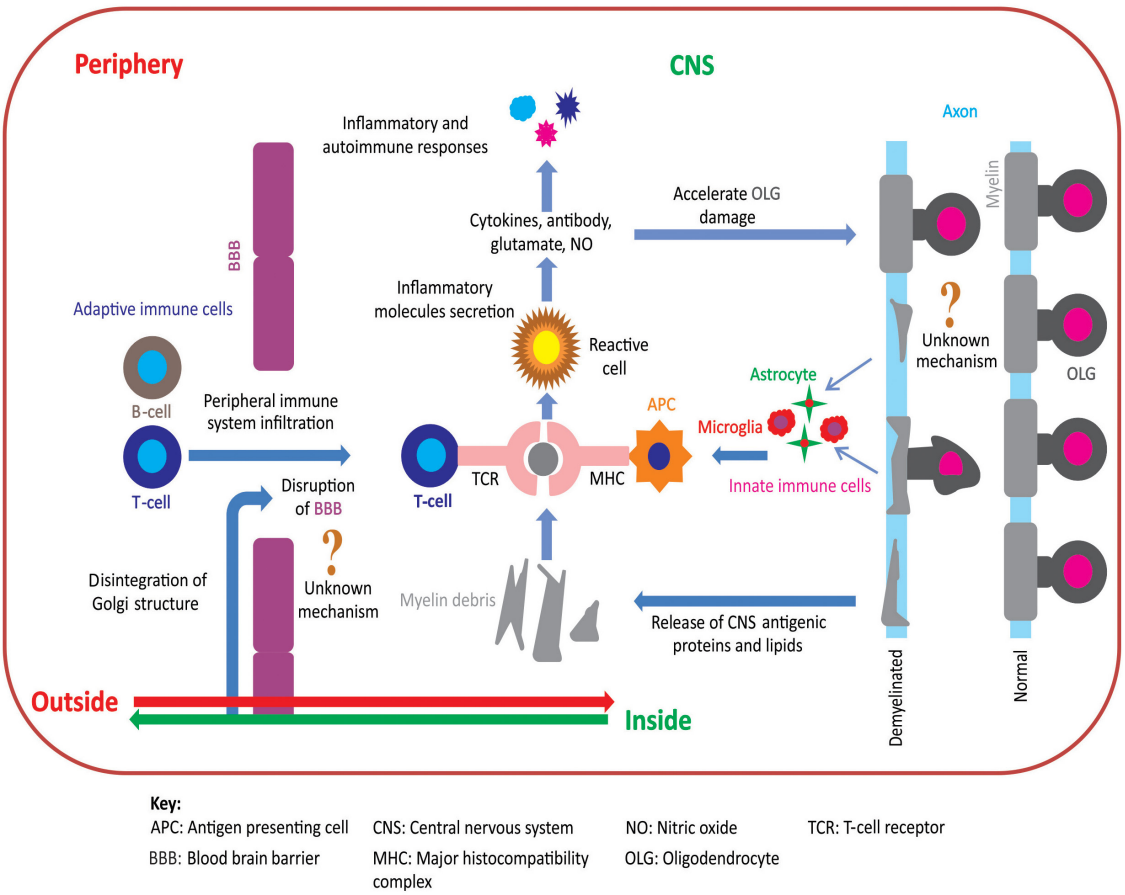

FIGURE 1 | Pathoetiology of oligodendrocytosis and autoimmunity in MS. There are two principal competing hypotheses of MS pathoetiology. In the "outside-in" hypothesis, an unknown trigger activates peripheral T- and/or B-cells and leads to an infiltration of these cells into the CNS via an apparently dysfunctional blood brain barrier (BBB). T-cells attack myelin in the CNS causing oligodendrocytes to degenerate, resulting in myelin loss and the release of myelin debris. Innate immune cells (e.g., microglia) engulf the myelin debris and act as antigen presenting cells to T-cells that then exacerbate the process of oligodendrocyte damage and demyelination. In contrast, in the "inside-out" hypothesis, oligodendrocyte degeneration is initially triggered by internal metabolic dysfunction in the CNS leading to demyelination and gliosis with subsequent release of inflammatory cytokines and chemokines. This cascade of events compromises the integrity of the BBB resulting in permeability to peripherally circulating T- and B-cells. Within the CNS, T-cells ($CD4^+$ and $CD8^+$) interact with antigen presenting cells, via major histocompatibility complex, and become activated. These activated T-cells also release inflammatory mediators such as cytokines, nitric oxide, and glutamate, which exacerbate the degenerative process leading to a subsequent immune response which further accelerates oligodendrocytosis and demyelination [adapted from (11)]. Figure was constructed using CorelDraw-version 2018 (www.coreldraw.com, Ottawa, ON, Canada) image processing software.

antibodies react remain poorly defined and are *not* disease-specific (4). For example, oligoclonal bands can be found in patients with other diseases such as neurosyphilis (5), subacute sclerosing panencephalitis (6), and falciparum malaria (7). Furthermore, it is not unusual for patients to be diagnosed with MS in the absence of oligoclonal bands (8, 9). These observations suggest that demyelination is the primary criterion (disseminated in time and space) to diagnose MS patients and the involvement of immune cells in MS is not necessary for the diagnosis.

Two opposing hypotheses have been proposed to explain the pathoetiology of MS: "outside-in" and "inside-out" (10–12). **Figure 1** summarizes these hypotheses of MS pathoetiology. In this Perspective, we first outline these hypotheses, explain their compatibility to the pathoetiology of MS, and examine how well these hypotheses are aligned with the outcomes of pre-clinical and clinical research. Finally, we show how recent advances using the cuprizone (CPZ) animal model support the inside-out hypothesis of MS pathoetiology, and how this research can be used to further investigate the earliest stages of demyelination, as well as the subsequent involvement of the adaptive immune response in aggravating the demyelinating lesions.

"Outside-in" theorists propose that an undefined dysregulation of the peripheral immune system and BBB leads to an autoimmune response against myelin components in the CNS. The central concept of and support for this hypothesis have been extensively drawn from studies using an animal model of experimental autoimmune encephalomyelitis (EAE). The EAE model was developed from a sequence of observations. The initial concept of EAE came from several observations of clinical interventions. First, 1885 rabies vaccinations to humans were shown, by 1888, to cause paralysis (13). Likewise, in 1925, repeated inoculation of humans with rabbit spinal

cord homogenates was shown to cause paralysis (13). Later, these observations motivated neurologists and researchers to investigate the effect of inoculating live animals with CNS extracts [e.g., spinal cord homogenates; (13)]. In the original article "*Observations on attempts to produce acute disseminated encephalomyelitis in monkeys*," the introduction revealed prevailing concerns about neurological disease and immunization and prophetically noted that "*The etiology of this malady is unknown in spite of the fact that considerable work has been done to disclose it*" (14). This study demonstrated that peripheral inoculation of monkeys with whole rabbit brain homogenate (injected intramuscularly) can induce a central glial reaction, loss of myelin sheaths, and "*cellular infiltration into the CNS*" (14). By 1934, a "*specific antigenicity of homologous brain runs parallel to the myelin content of the tissue*" was described and led to the speculation of post-infection encephalitis (15). In 1947, peripheral inoculation with brain homogenates in rabbit and rhesus monkey when combined with complete Freund adjuvant [paraffin oil plus heat-killed tubercle bacilli; (16)] resulted in a distribution of lesions with a "*distinct resemblance to that in disseminated encephalomyelitis and multiple sclerosis of human beings*" (17). It was also demonstrated that "*the perivascular position of the lesions, more particularly about venules and veins, is much like that in disseminated encephalomyelitis*" (17). Notably, the occurrence of relapse and remission cycles were similar to those seen in human MS (17). This model then underwent a progressive renaming from allergic encephalomyelitis (18) to EAE following the identification of the peripheral immunogenic role of exogenous human and guinea pig myelin basic protein (19, 20). The immunogenic/antigenic role of myelin basic protein may account for the over-/misinterpretation of the EAE model that has led to the widespread correlation of this model with MS rather than acute disseminated encephalomyelitis (ADEM). The correlation between EAE and the autoimmune aspects of MS has generated over 13,000 articles (https://pubmed.ncbi.nlm.nih.gov/2020) in the current form of EAE (mainly in mice) that relies on peripheral immunization with exogenous (human) antigens (e.g., myelin basic protein). Proponents of the EAE model, particularly with regard to immune cell involvement, have thus focused extensively (if not almost exclusively) on the immunological elements and argued for an (almost purely) immunological pathoetiology of MS [e.g., (21–23)]. Moreover, there is limited evidence (24) in EAE as to how autoantigens that originate in the CNS are recognized by the peripheral immune system. However, recent evidence indicates that CNS inflammation in EAE may be regulated by the meningeal lymphatic vasculature (24). Meningeal lymphatics assist in the drainage of cerebrospinal fluid-derived soluble molecules and immune cells into the lymph nodes. The ablation of meningeal lymphatics reduces the inflammatory response of brain-reactive T-cells in EAE, suggesting that drainage contributes to the activation of encephalitogenic T-cells in the lymph nodes (24). The selectivity of the immunological response to myelin elements within the CNS was questioned in studies showing that EAE also resulted in disruption of myelin structure in the peripheral nervous system and induced changes in sensory-motor functions (25–28). However, in MS, peripheral nerve involvement is limited to ∼5% of patients with chronic inflammatory polyneuropathy that is responsive to corticosteroid therapy (29). In this regard, the cross-reactivity of EAE with myelin in the peripheral and central nervous systems, which resembles only a very small subset of MS patients, may well reflect immunological aspects that are shared between EAE and ADEM rather than reflecting the pathoetiology of MS *per se*.

Arguments in support of the outside-in hypothesis have focused on the presence of T-cells, activated glial cells, and focal demyelination (types I–II, **Box 1**) in autopsy specimens from patients with *advanced* MS (30). It thus remains uncertain whether or not the focus on the immune aspects actually addresses the *primary* causes of disease initiation (i.e., pathoetiology), as inflammatory cells are evident in both demyelinated and non-demyelinated sites, indicating that the presence of inflammatory cells alone is insufficient to induce demyelination (31). Likewise, a detailed histological analysis of MS lesions demonstrated that "*1/3* of *CNS brain lesions did not involve peripheral inflammatory cells such as T-cells*" suggesting

BOX 1 | Key summary of the heterogeneity of MS lesions in the CNS based on oligodendrocytes loss, immune cell involvement, demyelination, and BBB disruption using histological examination [adapted from (30)].

Patterns I and II:

Similarities:

- Disruption of BBB which is confirmed by the presence of immunoglobulin G
- Plaques are centered by small veins and demarcated edges with peri-venous extensions
- Presence of remyelination shadow plaques (incomplete remyelinated lesions)
- Presence of T-lymphocytes and macrophages
- Less oligodendrocyte damage than in patterns III and IV
- A similar pattern of loss of myelin proteins (e.g., myelin basic protein)

Differences:

- Pattern I is found mainly in acute MS (patients who die or are subjected to biopsy within the first year after disease onset) whereas pattern II occurs mostly in chronic, relapsing-remitting MS, secondary progressive MS, and primary progressive MS
- Deposition of complement complex (part of the immune system promoting phagocytosis and inflammation) is found only in pattern II lesions

Patterns III and IV:

Similarities:

- Absence of remyelination shadow plaque
- Presence of T-lymphocytes and macrophages
- Large numbers of oligodendrocytes damaged
- Demyelinating lesions are around inflamed blood vessels
- Absence of immunoglobulin G and complement complex

Differences:

- Preferential loss of myelin-associated glycoprotein in pattern III
- Pattern III lesions are mostly found in patients with acute MS whereas pattern IV is found in primary progressive MS with prominent cerebellar and brain stem involvement.

an alternative pathoetiology of inflammation and demyelination (30). Specifically, MS lesions were characterized by extensive oligodendrocyte dystrophy and glial activation with little or no lymphocyte involvement, as seen in type III–IV lesions [**Box 1**; (30)]. Likewise, autopsy samples from a highly progressive MS patient (Marburg type) showed marked demyelination and glial activation in the CNS (32). In contrast, subtle perivascular infiltration of lymphocytes in the CNS and detection of few lymphocytes in the cerebrospinal fluid without any oligoclonal band were found in a 27-year-old woman (32). Additionally, autopsy samples from patients with acute MS showed nominal T- and B-cell involvement in the lesions, yet with marked oligodendrocyte loss and glial activation (33). Although MS lesions are heterogeneous (e.g., early, late, acute, or chronic), the staging or timing of MS lesion initiation and progression remains elusive (34).

These observations indicated that oligodendrocyte loss preceded the involvement of autoreactive T- or B-cells in the lesion site, i.e., *oligodendrocyte loss is an early disease event that can trigger (or otherwise be followed by) a subsequent autoimmune response*. Moreover, these observations highlighted the question as to what aspect of MS is being studied in EAE models given that the autoimmune response in humans occurs spontaneously, whereas in EAE the immune response is initiated in the periphery by the acute administration of relatively large amounts of exogenous antigens and immune activators. Additionally, there are notable differences in the cellular immune responses of MS and EAE: first, $CD8^+$ T-cells are the predominant immune cells in the CNS lesions of MS patients whereas, in EAE, $CD4^+$ T-cells predominate. Second, the demyelinating lesions in EAE are mainly located in the spinal cord, whereas in MS patients, lesions are mainly in the cerebral and cerebellar cortices [reviewed in (35–37)]. Third, there is a limited overlap of proteins and genes identified as being involved in EAE and MS (38, 39). Fourth, when ablation of the immune system in MS patients was followed by autologous haematopoietic stem cell transplantation, a reduction in the autoimmune response was observed but disease progression continued (40). That same study also demonstrated that over 74% of MS lesions in patients who received autologous stem cell transplantation had an active or complete demyelination with <26% of lesions showing signs of remyelination (40); furthermore, there was little T- or B-cell involvement indicating that these lesions did not originate due to an autoimmune response. However, recent work using bone marrow transplants in patients with primary progressive MS and secondary progressive MS showed reduced neurological dysfunction, better survival rates [i.e., longer lives; (41)], and a significant reduction in relapses (42), but the disease still progresses. Moreover, transplant-related mortality and exacerbation of neurological disabilities are not uncommon (41, 43, 44).

For how long can these marked differences between EAE and MS be ignored before we question the merit of accepting a correlation between an acute, peripherally-induced immune response and central demyelination (as seen in EAE) as an effective model for the *pathoetiology* of MS? It has been taken *ipso facto* as proof that a similar process underpins the initiation of EAE and MS, rather than a demonstration that EAE models a later immunological response to an earlier (and likely longer-term) oligodendrocytosis (i.e., degeneration or loss of oligodendrocytes resulting in subsequent demyelination). The almost overwhelming focus on EAE has thus allowed the "*tail to wag the mouse*" rather than the more realistic circumstance in which effect follows cause. In this scenario, MS (unlike EAE) is not a disease that is initially mediated by the T-cells trafficking from the periphery but is *caused* by another mechanism, namely "*degeneration of oligodendrocytes*" (11, 45). This must be the target if we are to understand the origins of the disease and effectively target a potential cure, a task from which we may have been diverted by focusing on EAE.

Despite nine decades (1930–2020) of intensive research, EAE has neither revealed a reliable early biomarker for MS nor aided in the development of a therapeutic that fully and effectively halts MS. Notably, EAE has most certainly advanced our understanding of the autoimmune aspects of MS, albeit later in the "clinical" stages of the disease (22, 46). Thus, several medications (e.g., glatiramer acetate, mitoxantrone, natalizumab), that are used for the symptomatic treatment of MS were developed after showing promising effects in EAE (47). Nevertheless, while these immunosuppressive therapies are not without the risk of significant side-effects, they can reduce the severity of the autoimmune attacks in some patients (e.g., decreasing the number of relapses in relapsing-remitting MS). However, they have little or no effect during the progressive phase (e.g., primary progressive and secondary progressive MS) of the disease (48, 49) suggesting alternative mechanism(s) underpinning the pathoetiology and/or disease progression of MS.

Due to the inability of EAE to address the initial, underlying pathoetiology of MS, recent studies have instead focused on the "inside-out" hypothesis of MS (50–52). This hypothesis argues that MS is initiated in the CNS as a degeneration of oligodendrocytes that subsequently triggers an innate immune response involving microglia and astrocytes, which act as antigen-presenting cells. This likely slow (i.e., perhaps over decades) degradation of oligodendrocytes results in the release of antigenic myelin proteins, such as myelin basic protein, into the circulatory and lymphatic systems, that trigger a subsequent autoimmune response in which peripheral T- and/or B-cells are activated and migrate into the CNS (10, 11). The inside-out hypothesis is consistent with autopsy findings showing extensive oligodendrocyte loss in MS patients (disease duration ∼39 months) with histologically proven active lesions (30). But notably, at the early time points (acute lesions with disease duration ∼20 days) of disease presentation, few inflammatory parenchymal T- and B-cells were found at the active demyelinating sites in the brain stem of MS patients (33, 53). Qualitative and quantitative magnetic resonance imaging and histopathologic analysis of the brains of chronic (disease duration ∼20 years), secondary, and primary progressive MS patients showed demyelination, extensive axonal loss, and chronic gliosis rather than focal inflammation (54). In the early clinical stages of MS (e.g., 2 weeks to 8 months following clinical diagnosis), electron microscopic studies have shown that

myelin degeneration or abnormal changes start at the innermost layers of the myelin sheaths, even at sites that are remote to the inflammatory CNS lesions. This suggests that the process of demyelination is not inevitably linked to inflammation and immune autoreactivity (55). Additionally, inadvertent detection of multifocal periventricular or juxta-cortical lesions in patients with non-neurological symptoms such as tumor, intracerebro ventricular bleeds, or headaches indicated the presence of lesions prior to the onset of clinical symptoms of MS (56). Likewise, samples analyzed from live MS patients also revealed less association of T- or B-cells in MS as seen in post-mortem samples (57–65). For example, changes of metabolites (e.g., a 30–40% increase of choline lipid) in normal-appearing white matter from MS patients are observed (measured by magnetic resonance *spectroscopic* imaging) before demyelinating lesions are detected using magnetic resonance imaging (65). When cerebrospinal fluid, blood, tear, or urine samples from live MS patients were analyzed few T-cell markers were detected compared to a host of other protein changes (57–64). For example, marked changes in apolipoprotein, ceruloplasmin, creatinine kinase, superoxide dismutase formed a major cluster of metabolic pathway changes in MS patients (57–64).

A genome-wide association study from 1,470 MS cases, including ∼2.5 million single-nucleotide polymorphisms revealed no single genome-wide significance indicative of MS susceptibility loci influencing MS severity [International Multiple Sclerosis Genetics Consortium (66)]. However, bioinformatic (KEGG pathway) analysis showed the enrichment of genes such as Ptprd, Ywhag, or Ifna16 from different pathways including natural killer cell-mediated cytotoxicity and Wnt signaling [regulates key aspects of organogenesis, neural patterning, cell migration (67)] may contribute to the perturbation of not only immune function but also extend to oligodendrocyte degeneration [International Multiple Sclerosis Genetics Consortium; (66)]. For example, overexpression of interleukin-2 (68) or natural killer cells (69) has been shown to evoke oligodendrocyte degeneration. Moreover, dysregulation of the Wnt signaling cascade impedes oligodendrocyte differentiation and maturation resulting in impaired myelination (70, 71) by modifying energy metabolism (72). Likewise, another genome-wide association study from MS patients revealed dysregulation of genes involved in immune regulation and metabolism (73). In addition, neuronal DNA damage (measured using γH2A.X as a marker) leads to abnormal cell cycle re-entry of oligodendrocytes (using cyclin D1 as a cell cycle marker) resulting in their death in MS (74). This evidence suggests that oligodendrocyte degeneration in MS is more heterogeneous than currently perceived and both metabolic dysregulation and genetic predisposition may contribute to disease initiation and progression (75).

Radiological abnormalities (identified in T2-weighted brain magnetic resonance imaging as hyperintense foci) are detectable in clinically asymptomatic relatives (e.g., parents, siblings, or children) of MS patients, suggesting that shared susceptibilities can manifest as radiological markers (76) which may indicate structural changes in myelin and infiltration of immune cells (77, 78). While such individuals may be clinically asymptomatic, they do display tell-tail signs of changes in sensory functions, such as poorer vibration perception, suggesting that there are underlying functional changes (35, 76). Furthermore, longitudinal sampling in military personnel revealed that the serum level of neurofilament light chain (a marker of axonal degeneration) was significantly elevated up to 6 years prior to the onset of clinical MS symptoms (79). Likewise, a 30-month longitudinal study revealed axonal injury (measured by proton magnetic resonance *spectroscopy*) in the "normal-appearing" white matter where magnetic resonance imaging was unable to show any changes (80). The interplay between the axon and its supporting oligodendrocytes was evident when the loss of myelin-associated glycoprotein and apoptotic-like oligodendrocyte destruction (as seen in type III MS lesions, **Box 1**) were seen at the inner layers of the myelin sheath whereas the outer layers, which are more readily accessible to interact with the immune system, were unaffected (81). Overall, a substantial body of data indicates that inherent early and long-term oligodendrocyte malfunction is the primary driver of demyelination, rather than autoimmune responses.

In MS patients, it is clear that adaptive immune cells do play a key role in the longer-term, clinically-defined pathology of the disease. However, the most important question is whether these immune cells instigate the primary pathology (as in the outside-in hypothesis) or represent a normal immune response directed against a preceding oligodendrocytosis/cytodegeneration (as in the inside-out hypothesis). If the latter hypothesis is correct, then the key is to identify the underlying mechanism(s) of primary oligodendrocyte death (i.e., oligodendrocytosis). For now, this is still uncertain, but there is growing evidence arising from research using the non-immune CPZ model of a demyelination mechanism based primarily on metabolic susceptibility (50, 82, 83).

This model is generated by feeding CPZ to rodents, resulting in oligodendrocytosis and subsequent CNS demyelination and gliosis (35, 82, 84, 85). In this model, CPZ also induces atrophy of the peripheral immune organs (e.g., spleen and thymus) and thus reduced T-cell levels, thereby suppressing the functions of the adaptive immune system. Therefore, the demyelinated lesions in the CNS of the CPZ-fed animals do not involve adaptive immune cell [i.e., T-cell; (82)]. In addition, as the BBB remains intact in the CPZ model this further reduces the capability of T-cells to infiltrate the CNS (82, 86), although the lymphatic system remains functional. While some have interpreted this lack of adaptive immune involvement as a shortcoming of the CPZ model, this was largely based on the longstanding dogma developed from the use of the EAE model. In fact, what the CPZ model demonstrates in the first instance is very selective and progressive MS-like damage without the involvement of the peripheral immune system (35, 85, 87). The importance of this observation was nonetheless downplayed in the literature for quite some time. Nonetheless, the CPZ model has now been further refined using strategic modifications that either boost the immune system following cessation of CPZ-feeding (50) or protect the peripheral immune system (51) thereby resulting in progressive demyelination, local inflammation (micro/astrogliosis), and subsequent $CD8^+$ T-cell infiltration into the CNS (inside-out response) when the BBB is breached.

CPZ MODEL: RELEVANCE TO MODELING MS

It is commonly considered that mature oligodendrocytes are susceptible to CPZ toxicity that induces mitochondrial oxidative stress (82, 88–90). Some authors have argued that CPZ can be used as a model to explore the mechanisms involved in the later, rather than early, disease stages of MS (36, 91). However, we, and others, have reported that CPZ can be used to investigate both aspects of the disease. For example, when CPZ is fed for a short period or at a low dose (e.g., 0.1%), significant oligodendrocytosis, demyelination, and accelerated remyelination are observed (51, 82, 92–94). In addition, oligodendrocyte degeneration (95) and glial responses (96) can be observed before detectable demyelination in the CPZ model. However, prolonged CPZ-feeding (e.g., 0.2% for 12 or more weeks) leads to a progressive ablation of oligodendrocytes, massive demyelination, and axonal injury (97–99). These data suggest that CPZ can be used to investigate both early and progressive stages of MS by titrating the dose and duration of CPZ-feeding.

Although there is limited evidence of successful translation, unlike EAE, of a remyelinating drug tested in CPZ and approved for human use (100), encouragingly, a large number of therapeutics are now being tested in the CPZ model for generating drugs to promote remyelination. For example, in 2020, 25/70 published papers have investigated different medications (PubMed by July 2020). However, studies using the CPZ model to investigate drug development started just over a decade ago (101) compared to EAE where efforts began over 70 years ago (102). Some authors have argued that both EAE and CPZ are important for investigating the different aspects of MS disease (36, 91, 103). To develop therapeutics against the autoimmune response in MS, the EAE model may be used since it is immune-driven (22, 46), if we accept that the disease process is mediated by CD4 T-cells. In contrast, to promote new myelin formation (demyelination is proven as the hallmark of MS) and minimize progressive degeneration, use of CPZ would be the best choice (100). However, perhaps the more important point with CPZ is that it may better reflect the preclinical stages of the disease [or perhaps the milder form of MS (104)], and thus its potential contribution to identifying much earlier, fundamental drug targets that might fully halt the disease process (and that could thus also be used as an adjunct to the current immune-related therapeutics).

Oligodendrocyte degeneration is followed by demyelination and activation of microglia and astrocytes, resulting in gliosis. This gliosis is distributed throughout all parts of the CNS but it is most marked in the cerebrum and cerebellum compared to the brain stem and spinal cord (82, 84, 96, 105). However, a recent study demonstrated that CPZ-feeding does not induce the degeneration of mature oligodendrocytes in the spinal cord (84). With only three studies (84, 106, 107) examining the effects CPZ on the spinal cord, using varying techniques in different mouse strains it may well be premature to assert a single action. For example, 4-weeks of CPZ-feeding in C57BL/6 mice appeared to have no impact on the luxol fast blue staining and immunodetection of myelin basic protein but did reduce mitochondrial complex IV abundance in the spinal cord; gliosis was not investigated (106). In SJL mice, TUNEL-positive apoptotic cells were found in the white matter of the spinal cord with reduced NogoA (a mature oligodendrocytes marker) and myelin basic protein mRNA expression; whereas no changes was observed in C57BL/6 mice (107). However, none of the mouse strains showed demyelination and glial activation (107). In contrast, in our recent study, 5 weeks of CPZ-feeding was associated with astro- and microgliosis in both the gray and white matter of the spinal cord (84). While preliminary evidence of reduced myelin basic protein staining (unpublished data), suggested demyelination, this was not confirmed using Silver staining (84). Whether the limited demyelination in the spinal cord of the CPZ model is due to technical issues [e.g., saturation of Silver staining in the high-density tracts of the spinal cord (*vis-a-vis* low-density tracts where demyelination was readily shown) (84)] or the use of different staining methods (e.g., Silver, myelin basic protein) remains unclear.

The apparent limited demyelination in the spinal cord in CPZ, unlike that seen in EAE, is less consistent with MS in which spinal cord lesions are seen, but less so than in the brain (108–111). However, the detection of spinal cord demyelination in humans is technically demanding (due to the thin cord, cerebrospinal fluid, bone, fat) with conventional imaging techniques and may go undiagnosed during asymptomatic stages (109, 110, 112, 113). Moreover, differential pathological outcomes are found in different segments of the spinal cord of MS patients; for example, lesions are more common in the cervical (∼60%) than the thoracic or lumbar spinal cord (114, 115). In addition to brain and cerebellum lesions, we have shown demyelination and gliosis in the brain stem of CPZ-fed mice (84) which is also seen in newly forming demyelinating lesions in MS patients (33, 116).

In addition to demyelination and gliosis, we (84) and others (117) have shown comparable functional deficits as seen in human MS [reviewed in (35)]. The apparent absence of correlation between behavioral deficits and histological changes may well be attributed to an undue focus on the corpus callosum (35); our most recent study showed that early motor deficits are associated with changes in the spinal cord, brain stem, and cerebella and cortical pathways associated with sensory-motor function (84). A perhaps more controversial interpretation of the results with CPZ is that the lack of more profound physical manifestations as seen in MS indicates that the main clinical symptoms of MS are not primarily related to demyelination but rather to molecular/cellular alterations that have yet to be effectively characterized. Alternatively, it maybe that rodents are of limited utility in modeling the human disease. As always, correlations (notably with findings in animal models) are just that, and interpretations, as to "likely" causation, vary.

These observations indicate that there are regional differences in the CNS in terms of oligodendrocytosis and highlight the fact that oligodendrocytosis and inflammatory responses can occur independently of each other. However, the underlying susceptibility to the differential response of CPZ on glial cells is neither clearly understood nor been systematically investigated. The heterogeneity of the glial response may depend upon the

differential expression of type III neuregulin-1 (118) and Fyn (119). For example, loss of the non-receptor tyrosine kinase Fyn (a signaling molecule of the Src kinase family) causes hypomyelination (120) in the brain rather than the spinal cord (119). Likewise, mice haplo-insufficient for type III neuregulin-1 (a growth factor that promotes oligodendrocyte and Schwann cell development) showed reduced myelination in the corpus callosum (118). In contrast, no effect was observed in the optic nerve and spinal cord, further indicating regional differences in the regulation of OLG function and their susceptibility to injury (118). Whether the expression of Fyn or neuregulin-1 contributes to the regional heterogeneity of oligodendrocytosis in CPZ-fed animals remains untested.

Another possibility for these effects could be that different regions of the CNS have different subtypes of oligodendrocytes (types I–IV) based on biochemical profile and axon myelination (121). Most recently, RNAscope analysis showed 12 different subtypes of mature oligodendrocytes that were not only differentially distributed in the brain and spinal cord but also responded differently to injury (122). Likewise, single-cell RNA sequencing of oligodendrocyte lineage cells from 10 CNS regions (e.g., hippocampus, hypothalamus) revealed 13 distinct populations (123), suggesting the region-specific expression of oligodendrocyte lineage cells in the CNS. In addition, oligodendrocyte lineage cells from EAE spinal cord show overexpression of genes involved in antigen processing and presentation via major histocompatibility complex classes I and II (124). In contrast, oligodendrocyte progenitor cells are capable of phagocytosis and activate memory and effector CD4-positive T-cells (124)—suggesting an oligodendrocyte mediated immune response in EAE. Likewise, a similar result was found when demyelinated areas (e.g., normal-appearing white matter) were investigated using single-cell RNA sequencing from MS patients (125). This analysis showed a differential expression of RNA markers in MS patients which was either unique or enriched (125). For example, platelet-derived growth factor receptor A (pdgfra) is uniquely expressed in oligodendrocyte progenitor cells, whereas apolipoprotein E (apoE) is expressed in immune oligodendroglia (125). Whether these aforementioned factors contribute to the regional distribution of glial responses in the CPZ model requires future investigation.

Despite the marked CNS oligodendrocytosis, demyelination, and gliosis in CPZ-fed mice, no involvement of adaptive immune cells in the CNS lesions was found (82, 86). However, T-cell infiltration into the CNS was not evident even when the BBB was disrupted by injection of ethidium bromide, lysolecithin (86), or pertussis toxin (82). This indicated the presence of an alternative mechanism(s) in the adaptive immune cell response in the CPZ model, independent of BBB integrity or the lymphatic system. Recent studies have shown CPZ-induced suppression of the adaptive immune system (82, 126–129). A time-dependent reduction in the number of $CD4^+$ T-cells (∼50%) and an inability to detect upregulation of T-cells (using CD44 and CD69 as markers) were observed in the corpus callosum following CPZ-feeding (126). Likewise, longer observations (10 months after cessation of CPZ-feeding) did not reveal any T-cell infiltration into the CNS (52). However, it was hypothesized that CPZ might have a direct immunosuppressive effect on the adaptive immune cells (52). Other studies demonstrated that the involvement of adaptive immune cells in EAE and Theiler's murine encephalomyelitis was reduced following CPZ-feeding, resulting in a delay in the development of disease characteristics (130–132). Our recent findings have revealed that the size of the spleen, as well as its T-cell ($CD4^+$ and $CD8^+$) levels, were reduced in CPZ-fed animals, following both short and prolonged feeding (82), confirming previous observations (127–129). Moreover, using a top-down proteomic analytical approach, a decreased abundance of specific proteoforms (e.g., of leukocyte elastase inhibitor A, calcium/calmodulin-dependent protein kinase type II subunit alpha, and disulphide isomerase) known to be involved in T-cell function were identified (82, 133). Furthermore, a reduction of the abundance of complement protein (part of the immune system) was found in the peripheral blood mononuclear cells of CPZ-fed animals (133). These findings indicated that CPZ-induced peripheral immune system suppression would have to be overcome in order to fully address the inside-out hypothesis of MS.

The molecular basis by which CPZ-ingestion causes adaptive immune system suppression is unclear, but CPZ chelates copper, leading to dyshomeostasis of other essential ions such as iron, zinc, sodium, and manganese in organs such as the brain and liver (134–138). The reduction in T-cells following CPZ-feeding is not surprising since copper is required for the synthesis of interleukin-2, and decreased levels of interleukin-2 interfere with the growth and maturation of T-cells (139, 140). Beyond the suppressive effect of CPZ (e.g., reduction of T-cell number), studies have revealed that T-cell functionality relies on mitochondrial activity (141). In CPZ-fed mice, mitochondrial division is inhibited resulting in the formation of extremely enlarged "mega-mitochondria" in oligodendrocytes, hepatocytes, and thymocytes (128, 142–145). Mega-mitochondria formation is an abnormal process that can result in excessive amounts of reactive oxygen species which reduce adenosine triphosphate supplies leading to cellular energy failure (146). Moreover, CPZ interferes with the fission and fusion dynamics of mitochondria due to the reduction of abundance of dynamin 1 protein (82) leading to the progressive swelling of mitochondria (88), reduction of mitochondrial transmembrane potential (90), and reduction of nicotinamide adenine dinucleotide metabolism (147). In addition, gene expression analysis revealed marked mitochondrial gene changes in CPZ-fed mice (148). Likewise, a selective loss of mitochondrial complex IV was found in cerebellar Purkinje neurons following CPZ-feeding for 5 weeks (149). Moreover, a marked decrease in the activities of mitochondrial complexes I-III in the brain of CPZ-fed mice was also found (150). Furthermore, the addition and deletion of mitochondrial DNA have been shown in CPZ-fed rats (151). These changes in mitochondrial function are also supported by human MS studies; for example, microarray analysis of post-mortem motor cortices from MS patients revealed the downregulation of nuclear-encoded mitochondrial genes and decreased activity of mitochondrial respiratory chain complexes I and III (152). This is also further supported by the elevated level of mitochondrial stress markers in the serum of MS

patients (153). Moreover, a case report revealed that mutation in the DNA polymerase gamma gene is responsible for the changes in mitochondrial function and has been implicated in MS-like illness including ophthalmoplegia, ataxia, and cognitive impairment (154) – suggesting that MS may well originate as a disease of mitochondrial dysfunction (155, 156).

Furthermore, proteomic and bioinformatic analyses revealed marked changes in metabolic pathways associated with mitochondrial functions (82). Thus, dysregulation of mitochondrial processes may underlie the compromise of the peripheral immune system in the CPZ model (157). In addition, since oligodendrocytes depend upon mitochondria for energy (158), the compromised mitochondria following CPZ-feeding supply less energy to oligodendrocytes, thus triggering oligodendrocytosis (i.e., the suggested initial trigger of MS).

Can CPZ-induced immune system suppression be prevented? We have addressed this question recently (51, 82). CPZ induces atrophy of the peripheral immune organs such as the spleen, making it impossible to see whether or not T-cells can invade the CNS when the BBB is breached (82). To circumvent this problem, we used juvenile mice [to avoid age-related thymic involution (159, 160)] and fed them 0.1% CPZ for 2 weeks to overcome the normal CPZ-mediated adaptive immune system atrophy (51). Juvenile animals showed less splenic and thymic atrophy when fed with 0.1 or 0.2% CPZ (51) compared with young adult mice (82, 127–129). However, no CNS T-cell infiltration was seen after the BBB was breached using pertussis toxin (51). In a parallel study, we castrated the juvenile mice to completely prevent splenic and thymic atrophy (51), since it is known that castration overcomes androgen-dependent (age-related) thymic involution and maximizes adaptive immune cell maturation and function (161, 162). When we combined castration, 0.1% CPZ-feeding and pertussis toxin injection to juvenile mice for 2 weeks, CD8 T-cell infiltration into the CNS, in addition to demyelination and gliosis, was observed (51). The result was confirmed by western blotting, immunohistochemistry, and flow cytometry (51). This work concluded that "*$CD8^+$ T-cell recruitment into the CNS of CPZ-fed mice, albeit castrated male mice, provides a potential new variant of the CPZ model with which to explore the early events involved in CNS demyelinating diseases like MS*" when the BBB is compromised (51). In contrast, gonadally intact female mice showed the routinely observed CPZ-induced thymic and splenic atrophy, and no T-cell infiltration into the CNS (51). It is noteworthy, however, that MS is more prevalent (2–3-fold) in females than males (163) suggesting that the lack of T-cell involvement in the CPZ-fed female mice (51) may indicate that different mechanism(s) are involved in the pathoetiology of MS in males and females. Whether female hormones (e.g., estrogen and progesterone) play a role in CPZ outcome or T-cell infiltration into the CNS remains untested. However, the preferential presence of $CD8^+$ T-cells in this model more closely resembles human MS pathology since $CD8^+$ T-cells outnumber $CD4^+$ T-cells by 3-10-fold in MS patients (164, 165). The work of Almuslehi et al. (51) is supported by another recent observation in which the peripheral immune systems of CPZ-fed mice were boosted by peripheral injection of complete Freund's adjuvant, and the BBB was breached (50). In this model, CNS infiltration of $CD3^+$ T-cells (a pan T-cell marker) was evident, and was followed by a secondary demyelination and inflammatory process. This variant of the CPZ model has been termed "cuprizone autoimmune encephalitis." The origin of the encephalomyelitis was attributed to citrullination (50), a post-translational modification in which the amino acid arginine is converted to citrulline leading to conformational changes of the affected proteins (166). Citrullination of myelin basic protein is linked with lymphocyte infiltration and demyelination in the spinal cord in EAE (167) and MS lesions (168). Western blot analysis showed a shift in the molecular weight in the blot of peptidyl arginine deiminases which are similar in molecular weight to myelin basic protein (50). Altogether, the data indicate that metabolic dysregulation in the CNS can lead to a subsequent peripheral immune response in CPZ-fed mice.

What happens when adoptive myelin-reactive T-cells are transferred to the CPZ-fed animal? This question was addressed recently (169–171). T-cells were transferred from EAE mice into CPZ-fed mice via intraperitoneal injection and $CD4^+$ T-cells infiltrated the corpus callosum; delayed remyelination was observed, suggesting that T-cells promote continuous demyelination and slowed remyelination in CPZ-fed mice (170). This model was further developed by Kirby et al. (171) who demonstrated that the adoptive transfer of myelin-reactive T-effector cells influenced the properties and differentiation of oligodendrocyte precursor cells (171). This work also showed that oligodendrocyte precursor cell differentiation is reduced by both effector T-cells and interferon-γ overexpression by astrocytes (171). Moreover, oligodendrocyte precursor cells exposed to interferon-γ cross-present antigens to cytotoxic CD8 T-cells, leading to oligodendrocyte precursor cell degeneration (171). Similarly, peripheral immunization of myelin oligodendrocyte glycoprotein 35–55 peptide into CPZ-fed mice induced myelin autoreactive T-cell infiltration into the CNS (169). All these studies indicate the importance of brain-intrinsic degenerative cascades for immune cell recruitment and degeneration of oligodendrocytes. However, histological investigation mainly concentrated on the corpus callosum (170, 171) and no reports of either behavioral deficits or proteomic changes associated with this model were found in the literature, clearly indicating that further studies are required. Moreover, these studies (169–171) relied on a preactivated anti-myelin (e.g., myelin oligodendrocyte glycoprotein) T-cell mediated immune response (arguably another variant of EAE; i.e., outside-in) rather than endogenous myelin (50–52).

New CPZ model variants (50, 51) reflect both primary oligodendrocytosis followed by the production of endogenous antigens (e.g., myelin debris) and a subsequent adaptive immune response. Whether or not T-cells were functionally active or if these treatments resulted in behavioral deficits were not tested (50, 51) and should be part of the next studies using these models. However, the microenvironment that facilitates T-cell infiltration was also not assessed in these recent studies (50, 51, 82). For example, the role of pro-inflammatory cytokines (e.g., interleukins-1 and−6, tumor necrosis factor-α, and interferon-γ) from microglia and astrocytes in the process of T-cell infiltration should be investigated (172). This would test which cytokine(s) are responsible for the peripheral T-cell activation and migration into the CNS following castration and the breach of the BBB

in CPZ-fed mice. Moreover, Sen et al. recently found a number of proteoforms (e.g., of calreticulin and dynamin) that appeared to have arisen due to selective post-translational modifications following CPZ-feeding (82); however, the antigenicity of these proteoforms was not tested (82) as for the peptidyl arginine deiminases (50). While this study (82) used whole-brain samples to identify proteome changes, detailed proteomic analysis of tissue from defined regions of the CNS including the cerebellum, brain stem, and spinal cord may explain the temporal effects of CPZ.

The presence of an "oligodendrocytosis triggering immune response" in the CPZ model is also supported by studies from the diphtheria toxin model (52). In this model, targeted oligodendrocyte degeneration is achieved either via external administration (173, 174) or genetic manipulation (52, 175, 176) of diphtheria toxin in the rodent. However, oligodendrocytosis triggering immune responses depend upon the duration and nature of degeneration. For example, when animals are treated for a short period such as 4 (174), 6 (176), or 5–20 weeks (173), motor behavioral deficits, oligodendrocyte degeneration, glial activation, and axonal injury are observed, but no adaptive immune response occurred. On the contrary, longer incubation leads to immune-mediated oligodendrocyte degeneration (52). In this work (52), ∼30 weeks after recovering from oligodendrocyte loss and demyelination, a secondary disease progression was observed which included motor behavioral deficits, weight loss, demyelination, and axonal injury. Importantly, this late-onset disease was also associated with increased numbers of T-lymphocytes in the CNS and myelin oligodendrocyte glycoprotein-specific T-cells in lymphoid organs (52). These data suggest that progressive degeneration of oligodendrocytes triggers an adaptive autoimmune response against myelin (52) and this is arguably more consistent with the apparent slow progression to MS (i.e., the disease, like other neurodegenerative diseases, may initiate years before the condition is clinically diagnosed).

While the diphtheria toxin model shows the sensory-motor behavioral deficits (52, 173, 175–177), these deficits do not extend to cognitive, affective (anxiety) or visual modalities like those seen in MS and the CPZ animal model (35). Moreover, studies revealed diphtheria toxin-mediated oligodendrocyte death and gliosis in the absence of a peripheral autoimmune response (173, 176, 177). Whether this effect is due to the effect of diphtheria toxin on peripheral immune organs, such as the spleen or thymus remains untested. Furthermore, research from our lab (82, 133) and others (89, 129, 178, 179) revealed similarities and differences of proteomic changes in MS and CPZ that remain unquantified in the diphtheria toxin model. In addition, the novel approaches adopted in the past 5–10 years of combining CPZ and EAE (169–171) and diphtheria toxin-induced oligodendrocytosis (52, 173–177) compared to the over 70 years of research on EAE (14) and ∼60 years on CPZ (180) have yet to reveal their role in promoting a better understanding of the pathoetiology of MS.

Taken together, evidence from animal models (50–52) thus support the proposed initial inside-out pathoetiology of MS (10, 11). While with diphtheria toxin it takes ∼1 year to see adaptive immune cell recruitment (52), in the CPZ model, this adaptive immune cell recruitment to the site of demyelination and subsequent immune-mediated demyelination is seen as early as 2 weeks after the start of CPZ-feeding (50, 51). Ideally, while it is quite unequivocal to state that there is no perfect animal model (perhaps for any human diseases) that mimics the complete complexity of MS, the legitimate use of an animal model depends upon the research question to be addressed and a considered presentation of the findings that acknowledges the limitations of the model. Having said that, significant progress in our understanding of MS has been made using animal models and it is our consistent hope that healthy debate, as presented in this Perspective, leads to better and more revealing experiments.

CONCLUSIONS

This Perspective describes why the EAE model is widely used to study the late (autoimmune) aspects of MS. Since, by design, EAE supports the outside-in hypothesis, it is thus not an effective model to study MS pathoetiology and should not be the model of choice when experiments are designed to identify the initiating trigger(s) of MS. We thus argue that MS primarily originates from a slow, progressive oligodendrocyte degeneration caused by metabolic dysfunction that leads to subsequent reactive gliosis in the absence of adaptive immune cell response. The CPZ model supports the inside-out hypothesis of MS pathoetiology, and can be modified to also study CNS infiltration of peripheral immune cells. The CPZ model thus has the advantage of enabling the study of oligodendrocytosis in the absence (immuno-suppression) and presence (immuno-protection) of the peripheral immune system via titration of the dose and time. However, the full extent to which adaptations of the CPZ model mimic human MS pathology is still unclear but appropriate studies into the potential underlying/initiating pathological alterations are now possible. Initially, this will require further optimization of the new models (e.g., confirming the functionality of T-cells and testing for behavioral changes) and thus potential validation as appropriate systems for identifying novel early biomarkers and therapeutic targets to most effectively address and perhaps even cure MS.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MS and DM conceived the idea and drafted the manuscript. MA, PS, and JC reviewed the manuscript. JC initiated the MS research project at WSU upon which this paper builds. All authors approved the final version.

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REFERENCES

1. Carswell R. Illustrations of the Elementary Forms of Disease. London: Longman. (1838).
2. Clanet M. Jean-Martin Charcot. 1825 to 1893. *Int MS J.* (2008). 15:59–61.
3. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lan Neurol.* (2018) 17:162–73. doi: 10.1016/S1474-4422(17)30470-2
4. Winger RC, Zamvil SS. Antibodies in multiple sclerosis oligoclonal bands target debris. *Proc Natl Acad Sci USA.* (2016) 113:7696–8. doi: 10.1073/pnas.1609246113
5. Mazzarello P, Poloni M, Citterio A, Camana C, Ceroni M. Cerebrospinal fluid IgG changes in neurosyphilis after high-dose penicillin G treatment. *Arch Neurol.* (1987) 44:249. doi: 10.1001/archneur.1987.00520150005003
6. Mehta PD, Patrick BA, Thormar H. Identification of virus-specific oligoclonal bands in subacute sclerosing panencephalitis by immunofixation after isoelectric focusing and peroxidase staining. *J Clin Microbiol.* (1982) 16:985–7. doi: 10.1128/JCM.16.5.985-987.1982
7. Mturi N, Keir G, Maclennan CA, Ross A, Willis AC, Elford BC, et al. Cerebrospinal fluid studies in kenyan children with severe falciparum malaria. *Open Trop Med J.* (2008) 1:56–62. doi: 10.2174/1874315300801010056
8. Huttner HB, Schellinger PD, Struffert T, Richter G, Engelhorn T, Bassemir T, et al. MRI criteria in MS patients with negative and positive oligoclonal bands: equal fulfillment of Barkhof's criteria but different lesion patterns. *J Neurol.* (2009) 256:1121–5. doi: 10.1007/s00415-009-5081-y
9. Zeman AZ, Kidd D, McLean BN, Kelly MA, Francis DA, Miller DH, et al. A study of oligoclonal band negative multiple sclerosis. *J Neurol Neurosurg Psy.* (1996) 60:27–30. doi: 10.1136/jnnp.60.1.27
10. Stys PK. Pathoetiology of multiple sclerosis: are we barking up the wrong tree? *F1000prime Rep.* (2013) 5:20. doi: 10.12703/P5-20
11. Stys PK, Zamponi GW, van Minnen J, Geurts JJ. Will the real multiple sclerosis please stand up? *Nat Rev Neurosci.* (2012) 13:507–14. doi: 10.1038/nrn3275
12. Stys PK, Tsutsui S. Recent advances in understanding multiple sclerosis. *F1000 Res.* (2019) 8:2100. doi: 10.12688/f1000research.20906.1
13. Baxter AG. The origin and application of experimental autoimmune encephalomyelitis. *Nat Rev Immunol.* (2007) 7:904–12. doi: 10.1038/nri2190
14. Rivers TM, Sprunt DH, Berry GP. Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J Exp Med.* (1933) 58:39–53. doi: 10.1084/jem.58.1.39
15. Schwentker FF, Rivers TM. The antibody response of rabbits to injections of emulsions and extracts of homologous brain. *J Exp Med.* (1934) 60:559–74. doi: 10.1084/jem.60.5.559
16. Freund J, Stern ER, Pisani TM. Isoallergic encephalomyelitis and radiculitis in guinea pigs after one injection of brain and mycobacteria in water-in-oil emulsion. *J Immunol.* (1947) 57:179–94.
17. Kabat EA, Wolf A, Bezer AE. The rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants. *J Exp Med.* (1947) 85:117–30. doi: 10.1084/jem.85.1.117
18. Morgan IM. Allergic encephalomyelitis in monkeys in response to injection of normal monkey nervous tissue. *J Exp Med.* (1947) 85:131–40. doi: 10.1084/jem.85.1.131
19. Laatsch RH, Kies MW, Gordon S, Alvord EC Jr. The encephalomyelitic activity of myelin isolated by ultracentrifugation. *J Exp Med.* (1962). 115:777–88. doi: 10.1084/jem.115.4.777
20. Carnegie PR, McPherson TA, Robson GS. Experimental autoimmune encephalomyelitis. Digestion of basic protein of human myelin with cyanogen bromide and trypsin. *Immunology.* (1970) 19:55–63.
21. Glatigny S, Bettelli E. Experimental Autoimmune Encephalomyelitis (EAE) as animal models of multiple sclerosis (MS). *Cold Spring Harbor Pers Med.* (2018) 8:28977. doi: 10.1101/cshperspect.a028977
22. Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain.* (2006) 129:1953–71. doi: 10.1093/brain/awl075
23. Burrows DJ, McGown A, Jain SA, De Felice M, Ramesh TM, Sharrack B, et al. Animal models of multiple sclerosis: From rodents to zebrafish. *Mul Scl J.* (2018) 25:306–24. doi: 10.1177/1352458518805246
24. Louveau A, Herz J, Alme MN, Salvador AF, Dong MQ, Viar KE, et al. CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature. *Nat Neurosci.* (2018) 21:1380–91. doi: 10.1038/s41593-018-0227-9
25. Pender MP, Sears TA. Vulnerability of the dorsal root ganglion in experimental allergic encephalomyelitis. *Clin Exp Neurol.* (1985) 21:211–23.
26. Pender MP, Sears TA. Involvement of the dorsal root ganglion in acute experimental allergic encephalomyelitis in the lewis rat: a histological and electrophysiological study. *J Neurol Sci.* (1986) 72:231–42. doi: 10.1016/0022-510X(86)90011-0
27. Pender MP, Tabi Z, Nguyen KB, McCombe PA. The proximal peripheral nervous system is a major site of demyelination in experimental autoimmune encephalomyelitis induced in the Lewis rat by a myelin basic protein-specific T cell clone. *Acta Neuropathol.* (1995) 89:527–31. doi: 10.1007/BF00571507
28. Wang IC, Chung C-Y, Liao F, Chen C-C, Lee C-H. Peripheral sensory neuron injury contributes to neuropathic pain in experimental autoimmune encephalomyelitis. *Sci Rep.* (2017) 7:42304. doi: 10.1038/srep42304
29. Misawa S, Kuwabara S, Mori M, Hayakawa S, Sawai S, Hattori T. Peripheral nerve demyelination in multiple sclerosis. *Clin Neurophysiol.* (2008) 119:1829–33. doi: 10.1016/j.clinph.2008.04.010
30. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol.* (2000) 47:707–17. doi: 10.1002/1531-8249(200006)47:6<707::AID-ANA3>3.0.CO;2-Q
31. Ozawa K, Suchanek G, Breitschopf H, Brück W, Budka H, Jellinger K, et al. Patterns of oligodendroglia pathology in multiple sclerosis. *Brain.* (1994) 117:1311–22. doi: 10.1093/brain/117.6.1311
32. Wood DD, Bilbao JM, O'Connors P, Moscarello MA. Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. *Ann Neurol.* (1996) 40:18–24. doi: 10.1002/ana.410400106
33. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann Neurol.* (2004) 55:458–68. doi: 10.1002/ana.20016
34. van der Valk P, De Groot CJ. Staging of multiple sclerosis (MS) lesions: pathology of the time frame of MS. *Neuropathol App Neurobiol.* (2000) 26:2–10. doi: 10.1046/j.1365-2990.2000.00217.x
35. Sen MK, Mahns DA, Coorssen JR, Shortland PJ. Behavioural phenotypes in the cuprizone model of central nervous system demyelination. *Neurosci Biobehav Rev.* (2019) 107:23–46. doi: 10.1016/j.neubiorev.2019.08.008
36. Ransohoff RM. Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat Neurosci.* (2012) 15:1074–7. doi: 10.1038/nn.3168
37. Sriram S, Steiner I. Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. *Ann Neurol.* (2005) 58:939–45. doi: 10.1002/ana.20743
38. Farias AS, Pradella F, Schmitt A, Santos LM, Martins-de-Souza D. Ten years of proteomics in multiple sclerosis. *Proteomic.* (2014) 14:467–80. doi: 10.1002/pmic.201300268
39. Raddatz BBR, Hansmann F, Spitzbarth I, Kalkuhl A, Deschl U, Baumgärtner W, et al. Transcriptomic meta-analysis of multiple sclerosis and its experimental models. *PLoS ONE.* (2014) 9:e86643. doi: 10.1371/journal.pone.0086643
40. Metz I, Lucchinetti CF, Openshaw H, Garcia-Merino A, Lassmann H, Freedman MS, et al. Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. *Brain.* (2007) 130:1254–62. doi: 10.1093/brain/awl370
41. Muraro PA, Pasquini M, Atkins HL, Bowen JD, Farge D, Fassas A, et al. Long-term outcomes after autologous hematopoietic stem cell transplantation for multiple sclerosis. *JAMA Neurol.* (2017) 74:459–69. doi: 10.1001/jamaneurol.2016.5867
42. Burt RK, Balabanov R, Han X, Sharrack B, Morgan A, Quigley K, et al. Association of nonmyeloablative hematopoietic stem cell transplantation with neurological disability in patients with relapsing-remitting multiple sclerosis. *JAMA.* (2015) 313:275–84. doi: 10.1001/jama.2014.17986
43. Fassas A, Passweg JR, Anagnostopoulos A, Kazis A, Kozak T, Havrdova E, et al. Hematopoietic stem cell transplantation for multiple sclerosis.

A retrospective multicenter study. *J Neurol.* (2002) 249:1088–97. doi: 10.1007/s00415-002-0800-7
44. Kuan TLT, Amini F, Seghayat MS. Feasibility and toxicity of hematopoietic stem cell transplant in multiple sclerosis. *Iran J Bas Med Sci.* (2017) 20:729–38. doi: 10.22038/IJBMS.2017.9000
45. Partridge MA, Myers SJ, Gopinath S, Coorssen JR. Proteomics of a conundrum: thoughts on addressing the aetiology vs. progression of multiple sclerosis. *Proteomic Clin App.* (2015) 9:838–43. doi: 10.1002/prca.201400141
46. Krishnamoorthy G, Wekerle H. EAE: an immunologist's magic eye. *Eur J Immunol.* (2009) 39:2031–5. doi: 10.1002/eji.200939568
47. Steinman L, Zamvil SS. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. *Ann Neurol.* (2006) 60:12–21. doi: 10.1002/ana.20913
48. Buck D, Hemmer B. Treatment of multiple sclerosis: current concepts and future perspectives. *J Neurol.* (2011) 258:1747–62. doi: 10.1007/s00415-011-6101-2
49. Vargas DL, Tyor WR. Update on disease-modifying therapies for multiple sclerosis. *J Invest Med.* (2017) 65:883–91. doi: 10.1136/jim-2016-000339
50. Caprariello AV, Rogers JA, Morgan ML, Hoghooghi V, Plemel JR, Koebel A, et al. Biochemically altered myelin triggers autoimmune demyelination. *Proc Natl Acad Sci USA.* (2018) 115:5528–33. doi: 10.1073/pnas.1721115115
51. Almuslehi MSM, Sen MK, Shortland PJ, Mahns DA, Coorssen JR. CD8 T-cell recruitment into the central nervous system of cuprizone-fed mice: relevance to modeling the etiology of multiple sclerosis. *Front Cell Neurosci.* (2020) 14:43. doi: 10.3389/fncel.2020.00043
52. Traka M, Podojil JR, McCarthy DP, Miller SD, Popko B. Oligodendrocyte death results in immune-mediated CNS demyelination. *Nat Neusci.* (2016) 15:65–74. doi: 10.1038/nn.4193
53. Henderson AP, Barnett MH, Parratt JD, Prineas JW. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann Neurol.* (2009) 66:739–53. doi: 10.1002/ana.21800
54. Seewann A, Vrenken H, van der Valk P, Blezer EL, Knol DL, Castelijns JA, et al. Diffusely abnormal white matter in chronic multiple sclerosis: imaging and histopathologic analysis. *Arc Neurol.* (2009) 66:601–9. doi: 10.1001/archneurol.2009.57
55. Rodriguez M, Scheithauer B. Ultrastructure of multiple sclerosis. *Ultrastruc Path.* (1994) 18:3–13. doi: 10.3109/01913129409016267
56. Maia ACM, Jr., Rocha AJd, Barros BR, Tilbery CP. Incidental demyelinating inflammatory lesions in asymptomatic patients: a brazilian cohort with radiologically isolated syndrome and a critical review of current literature. *Arquivos Neuro Psiquiatria.* (2012) 70:5–11. doi: 10.1590/S0004-282X2012000100003
57. Comabella M, Fernandez M, Martin R, Rivera-Vallve S, Borras E, Chiva C, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain.* (2010) 133:1082–93. doi: 10.1093/brain/awq035
58. Dumont D, Noben JP, Raus J, Stinissen P, Robben J. Proteomic analysis of cerebrospinal fluid from multiple sclerosis patients. *Proteomic.* (2004) 4:2117–24. doi: 10.1002/pmic.200300715
59. Hammack BN, Fung KY, Hunsucker SW, Duncan MW, Burgoon MP, Owens GP, et al. Proteomic analysis of multiple sclerosis cerebrospinal fluid. *Mul Scl.* (2004) 10:245–60. doi: 10.1191/1352458504ms1023oa
60. Berge T, Eriksson A, Brorson IS, Hogestol EA, Berg-Hansen P, Doskeland A, et al. Quantitative proteomic analyses of CD4(+) and CD8(+) T cells reveal differentially expressed proteins in multiple sclerosis patients and healthy controls. *Clin Proteomic.* (2019) 16:19. doi: 10.1186/s12014-019-9241-5
61. Rithidech KN, Honikel L, Milazzo M, Madigan D, Troxell R, Krupp LB. Protein expression profiles in pediatric multiple sclerosis: potential biomarkers. *Mul Scl.* (2009) 15:455–64. doi: 10.1177/1352458508100047
62. Salvisberg C, Tajouri N, Hainard A, Burkhard PR, Lalive PH, Turck N. Exploring the human tear fluid: discovery of new biomarkers in multiple sclerosis. *Proteomic Clin App.* (2014) 8:185–94. doi: 10.1002/prca.201300053
63. De Masi R, Vergara D, Pasca S, Acierno R, Greco M, Spagnolo L, et al. PBMCs protein expression profile in relapsing IFN-treated multiple sclerosis: a pilot study on relation to clinical findings and brain atrophy. *J Neuroimmunol.* (2009) 210:80–6. doi: 10.1016/j.jneuroim.2009.03.002
64. Singh V, Stingl C, Stoop MP, Zeneyedpour L, Neuteboom RF, Smitt PS, et al. Proteomics urine analysis of pregnant women suffering from multiple sclerosis. *J Proteome Res.* (2015) 14:2065–73. doi: 10.1021/pr501162w
65. Narayana PA, Doyle TJ, Lai D, Wolinsky JS. Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. *Ann Neurol.* (1998) 43:56–71. doi: 10.1002/ana.410430112
66. Genome-wide association study of severity in multiple sclerosis. *Gene Immun.* (2011) 12:615–25. doi: 10.1038/gene.2011.34
67. Komiya Y, Habas R. Wnt signal transduction pathways. *Organogene.* (2008) 4:68–75. doi: 10.4161/org.4.2.5851
68. Morse RH, Séguin R, McCrea EL, Antel JP. NK cell-mediated lysis of autologous human oligodendrocytes. *J Neuroimmunol.* (2001) 116:107–15. doi: 10.1016/S0165-5728(01)00289-2
69. Zaguia F, Saikali P, Ludwin S, Newcombe J, Beauseigle D, McCrea E, et al. Cytotoxic NKG2 CD4 T cells target oligodendrocytes in multiple sclerosis. *J Immunol.* (2013) 190:2510–8. doi: 10.4049/jimmunol.1202725
70. Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes Develop.* (2009) 23:1571–85. doi: 10.1101/gad.1806309
71. Feigenson K, Reid M, See J, Crenshaw EB 3rd, Grinspan JB. Wnt signaling is sufficient to perturb oligodendrocyte maturation. *Mol Cell Neurosci.* (2009) 42:255–65. doi: 10.1016/j.mcn.2009.07.010
72. Vallée A, Lecarpentier Y, Guillevin R, Vallée JN. Demyelination in multiple sclerosis: reprogramming energy metabolism and potential pparγ agonist treatment approaches. *Int J Mol Sci.* (2018) 19:1212. doi: 10.3390/ijms19041212
73. Liu G, Zhang F, Jiang Y, Hu Y, Gong Z, Liu S, et al. Integrating genome-wide association studies and gene expression data highlights dysregulated multiple sclerosis risk pathways. *Mul Scl.* (2017) 23:205–12. doi: 10.1177/1352458516649038
74. Tse KH, Cheng A, Ma F, Herrup K. DNA damage-associated oligodendrocyte degeneration precedes amyloid pathology and contributes to Alzheimer's disease and dementia. *Alz Demen.* (2018) 14:664–79. doi: 10.1016/j.jalz.2017.11.010
75. Young CA. Factors predisposing to the development of multiple sclerosis. *QJM.* (2011) 104:383–6. doi: 10.1093/qjmed/hcr012
76. Xia Z, Steele SU, Bakshi A, Clarkson SR, White CC, Schindler MK, et al. Assessment of early evidence of multiple sclerosis in a prospective study of asymptomatic high-risk family members. *JAMA Neurol.* (2017) 74:293–300. doi: 10.1001/jamaneurol.2016.5056
77. Absinta M, Sati P, Fechner A, Schindler MK, Nair G, Reich DS. Identification of chronic active multiple sclerosis lesions on 3T MRI. *AJNR Amn J Neuroradiol.* (2018) 39:1233–8. doi: 10.3174/ajnr.A5660
78. Fox RJ, Beall E, Bhattacharyya P, Chen JT, Sakaie K. Advanced MRI in multiple sclerosis: current status and future challenges. *Neurol Clin.* (2011) 29:357–80. doi: 10.1016/j.ncl.2010.12.011
79. Bjornevik K, Munger KL, Cortese M, Barro C, Healy BC, Niebuhr DW, et al. Serum neurofilament light chain levels in patients with presymptomatic multiple sclerosis. *JAMA Neurol.* (2019) 2019:e193238. doi: 10.1001/jamaneurol.2019.3238
80. De Stefano N, Matthews PM, Fu L, Narayanan S, Stanley J, Francis GS, et al. Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain.* (1998) 121:1469–77. doi: 10.1093/brain/121.8.1469
81. Aboul-Enein F, Rauschka H, Kornek B, Stadelmann C, Stefferl A, Brück W, et al. Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. *J Neuropathol Exp Neurol.* (2003) 62:25–33. doi: 10.1093/jnen/62.1.25
82. Sen MK, Almuslehi MSM, Gyengesi E, Myers SJ, Shortland PJ, Mahns DA, et al. Suppression of the peripheral immune system limits the central immune response following cuprizone-feeding: relevance to modelling multiple sclerosis. *Cells.* (2019) 8:1314. doi: 10.3390/cells8111314
83. Caprariello AV, Stys PK. Turned inside out: will myelin-protective therapies become the next-generation anti-inflammatories? *DNA Cell Biol.* (2019) 38:219–22. doi: 10.1089/dna.2018.4496

84. Sen MK, Almuslehi MSM, Coorssen JR, Mahns DA, Shortland PJ. Behavioural and histological changes in cuprizone-fed mice. *Brain Behav Immun.* (2020) 87:508–23. doi: 10.1016/j.bbi.2020.01.021
85. Praet J, Guglielmetti C, Berneman Z, Van der Linden A, Ponsaerts P. Cellular and molecular neuropathology of the cuprizone mouse model: clinical relevance for multiple sclerosis. *Neurosci Biobev Rev.* (2014) 47:485–505. doi: 10.1016/j.neubiorev.2014.10.004
86. Tejedor LS, Wostradowski T, Gingele S, Skripuletz T, Gudi V, Stangel M. The effect of stereotactic injections on demyelination and remyelination: a study in the cuprizone model. *J Mol Neurosci.* (2017) 61:479–88. doi: 10.1007/s12031-017-0888-y
87. Kipp M, Clarner T, Dang J, Copray S, Beyer C. The cuprizone animal model: new insights into an old story. *Acta Neuropathol.* (2009) 118:723–36. doi: 10.1007/s00401-009-0591-3
88. Faizi M, Salimi A, Seydi E, Naserzadeh P, Kouhnavard M, Rahimi A, et al. Toxicity of cuprizone a Cu(2+) chelating agent on isolated mouse brain mitochondria: a justification for demyelination and subsequent behavioral dysfunction. *Toxicol Mech Meth.* (2016) 26:276–83. doi: 10.3109/15376516.2016.1172284
89. Werner SR, Saha JK, Broderick CL, Zhen EY, Higgs RE, Duffin KL, et al. Proteomic analysis of demyelinated and remyelinating brain tissue following dietary cuprizone administration. *J Mol Neurosci.* (2010) 42:210–25. doi: 10.1007/s12031-010-9354-9
90. Benardais K, Kotsiari A, Skuljec J, Koutsoudaki PN, Gudi V, Singh V, et al. Cuprizone [bis(cyclohexylidenehydrazide)] is selectively toxic for mature oligodendrocytes. *Neurotox Res.* (2013) 24:244–50. doi: 10.1007/s12640-013-9380-9
91. Faissner S, Plemel JR, Gold R, Yong VW. Progressive multiple sclerosis: from pathophysiology to therapeutic strategies. *Nat Rev Drug Discover.* (2019) 18:905–22. doi: 10.1038/s41573-019-0035-2
92. Zhen W, Liu A, Lu J, Zhang W, Tattersall D, Wang J. An alternative cuprizone-induced demyelination and remyelination mouse model. *ASN Neurol.* (2017) 9:1759091417725174. doi: 10.1177/1759091417725174
93. Pfeifenbring S, Nessler S, Wegner C, Stadelmann C, Bruck W. Remyelination after cuprizone-induced demyelination is accelerated in juvenile mice. *J Neuropathol Exp Neurol.* (2015) 74:756–66. doi: 10.1097/NEN.0000000000000214
94. Steelman AJ, Thompson JP, Li J. Demyelination and remyelination in anatomically distinct regions of the corpus callosum following cuprizone intoxication. *Neurosci Res.* (2012) 72:32–42. doi: 10.1016/j.neures.2011.10.002
95. Mason JL, Jones JJ, Taniike M, Morell P, Suzuki K, Matsushima GK. Mature oligodendrocyte apoptosis precedes IGF-1 production and oligodendrocyte progenitor accumulation and differentiation during demyelination/remyelination. *J Neurosci Res.* (2000) 61:251–62. doi: 10.1002/1097-4547(20000801)61:3<251::AID-JNR3>3.0.CO;2-W
96. Hiremath MM, Saito Y, Knapp GW, Ting JP, Suzuki K, Matsushima GK. Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *J Neuroimmunol.* (1998) 92:38–49. doi: 10.1016/S0165-5728(98)00168-4
97. Hibbits N, Yoshino J, Le TQ, Armstrong RC. Astrogliosis during acute and chronic cuprizone demyelination and implications for remyelination. *ASN Neurol.* (2012) 4:393–408. doi: 10.1042/AN20120062
98. Skripuletz T, Lindner M, Kotsiari A, Garde N, Fokuhl J, Linsmeier F, et al. Cortical demyelination is prominent in the murine cuprizone model and is strain-dependent. *Amn J Pathol.* (2008) 172:1053–61. doi: 10.2353/ajpath.2008.070850
99. Sun SW, Liang HF, Trinkaus K, Cross AH, Armstrong RC, Song SK. Noninvasive detection of cuprizone induced axonal damage and demyelination in the mouse corpus callosum. *Mag Res Med.* (2006) 55:302–8. doi: 10.1002/mrm.20774
100. Hooijmans CR, Hlavica M, Schuler FAF, Good N, Good A, Baumgartner L, et al. Remyelination promoting therapies in multiple sclerosis animal models: a systematic review and meta-analysis. *Sci Rep.* (2019) 9:822. doi: 10.1038/s41598-018-35734-4
101. Torkildsen O, Brunborg LA, Milde AM, Mork SJ, Myhr KM, Bo L. A salmon based diet protects mice from behavioural changes in the cuprizone model for demyelination. *Clinic Nut.* (2009) 28:83–7. doi: 10.1016/j.clnu.2008.10.015
102. Gooo RA, Campbell B, Good TA. Prophylactic and therapeutic effect of para-aminobenzoic acid and sodium salicylate on experimental allergic encephalomyelitis. *Proc Soc Exp Biol Med.* (1949) 72:341–7. doi: 10.3181/00379727-72-17426
103. Kipp M, van der Star B, Vogel DY, Puentes F, van der Valk P, Baker D, et al. Experimental *in vivo* and *in vitro* models of multiple sclerosis: EAE and beyond. *Mul Scl Rel Dis.* (2012) 1:15–28. doi: 10.1016/j.msard.2011.09.002
104. Sorensen PS, Sellebjerg F, Hartung H-P, Montalban X, Comi G, Tintoré M. The apparently milder course of multiple sclerosis: changes in the diagnostic criteria, therapy and natural history. *Brain.* (2020) 145. doi: 10.1093/brain/awaa145. [Epub ahead of print].
105. Goldberg J, Clarner T, Beyer C, Kipp M. Anatomical distribution of cuprizone-induced lesions in C57BL6 mice. *J Mol Neurosci.* (2015) 57:166–75. doi: 10.1007/s12031-015-0595-5
106. Acs P, Selak MA, Komoly S, Kalman B. Distribution of oligodendrocyte loss and mitochondrial toxicity in the cuprizone-induced experimental demyelination model. *J Neuroimmunol.* (2013) 262:128–31. doi: 10.1016/j.jneuroim.2013.06.012
107. Herder V, Hansmann F, Stangel M, Skripuletz T, Baumgartner W, Beineke A. Lack of cuprizone-induced demyelination in the murine spinal cord despite oligodendroglial alterations substantiates the concept of site-specific susceptibilities of the central nervous system. *Neuropathol App Neurobiol.* (2011) 37:676–84. doi: 10.1111/j.1365-2990.2011.01168.x
108. Nociti V, Cianfoni A, Mirabella M, Caggiula M, Frisullo G, Patanella AK, et al. Clinical characteristics, course and prognosis of spinal multiple sclerosis. *Spinal Cord.* (2005) 43:731–4. doi: 10.1038/sj.sc.3101798
109. Ciccarelli O, Cohen JA, Reingold SC, Weinshenker BG. Spinal cord involvement in multiple sclerosis and neuromyelitis optica spectrum disorders. *Lan Neurol.* (2019) 18:185–97. doi: 10.1016/S1474-4422(18)30460-5
110. Marrodan M, Gaitán MI, Correale J. Spinal cord involvement in ms and other demyelinating diseases. *Biomedichine.* (2020) 8:130. doi: 10.3390/biomedicines8050130
111. Love S. Demyelinating diseases. *J Clin Pathol.* (2006) 59:1151–9. doi: 10.1136/jcp.2005.031195
112. Barakat N, Gorman MP, Benson L, Becerra L, Borsook D. Pain and spinal cord imaging measures in children with demyelinating disease. *NeuroImage Clin.* (2015) 9:338–47. doi: 10.1016/j.nicl.2015.08.019
113. Stroman PW, Wheeler-Kingshott C, Bacon M, Schwab JM, Bosma R, Brooks J, et al. The current state-of-the-art of spinal cord imaging: methods. *NeuroImage.* (2014) 84:1070–81. doi: 10.1016/j.neuroimage.2013.04.124
114. DeLuca GC, Williams K, Evangelou N, Ebers GC, Esiri MM. The contribution of demyelination to axonal loss in multiple sclerosis. *Brain.* (2006) 129:1507–16. doi: 10.1093/brain/awl074
115. Moccia M, Ruggieri S, Ianniello A, Toosy A, Pozzilli C, Ciccarelli O. Advances in spinal cord imaging in multiple sclerosis. *Therap Adv Neurol Dis.* (2019) 12:1756286419840593. doi: 10.1177/1756286419840593
116. Adams CWM, Poston RN, Buk SJ. Pathology, histochemistry and immunocytochemistry of lesions in acute multiple sclerosis. *J Neurol Sci.* (1989) 92:291–306. doi: 10.1016/0022-510X(89)90144-5
117. Franco-Pons N, Torrente M, Colomina MT, Vilella E. Behavioral deficits in the cuprizone-induced murine model of demyelination/remyelination. *Toxicol Let.* (2007) 169:205–13. doi: 10.1016/j.toxlet.2007.01.010
118. Taveggia C, Thaker P, Petrylak A, Caporaso GL, Toews A, Falls DL, et al. Type III neuregulin-1 promotes oligodendrocyte myelination. *Glia.* (2008) 56:284–93. doi: 10.1002/glia.20612
119. Sperber BR, Boyle-Walsh EA, Engleka MJ, Gadue P, Peterson AC, Stein PL, et al. A unique role for Fyn in CNS myelination. *J Neurosci.* (2001) 21:2039–47. doi: 10.1523/JNEUROSCI.21-06-02039.2001
120. Umemori H, Sato S, Yagi T, Aizawa S, Yamamoto T. Initial events of myelination involve Fyn tyrosine kinase signalling. *Nature.* (1994) 367:572–6. doi: 10.1038/367572a0
121. Butt AM, Ibrahim M, Ruge FM, Berry M. Biochemical subtypes of oligodendrocyte in the anterior medullary velum of the rat as revealed by the monoclonal antibody Rip. *Glia.* (1995) 14:185–97. doi: 10.1002/glia.440140304

122. Floriddia E, Zhang S, van Bruggen D, Gonçalves dos Santos J, Altinkök M, Llorens-Bobadilla E, et al. Distinct oligodendrocyte populations have spatial preference and injury-specific responses. *bioRxiv [Preprint].* (2019) doi: 10.1101/580985
123. Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcao A, Xiao L, et al. Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Sci.* (2016) 352:1326–9. doi: 10.1126/science.aaf6463
124. Falcão AM, van Bruggen D, Marques S, Meijer M, Jäkel S, Agirre E, et al. Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. *Nat Med.* (2018) 24:1837–44. doi: 10.1038/s41591-018-0236-y
125. Jäkel S, Agirre E, Mendanha Falcão A, van Bruggen D, Lee KW, Knuesel I, et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature.* (2019) 566:543–7. doi: 10.1038/s41586-019-0903-2
126. Remington LT, Babcock AA, Zehntner SP, Owens T. Microglial recruitment, activation, and proliferation in response to primary demyelination. *Amn J Pathol.* (2007) 170:1713–24. doi: 10.2353/ajpath.2007.060783
127. Sui RX, Miao Q, Wang J, Wang Q, Song LJ, Yu JW, et al. Protective and therapeutic role of Bilobalide in cuprizone-induced demyelination. *Int Immunopharmacol.* (2018) 66:69–81. doi: 10.1016/j.intimp.2018.09.041
128. Solti I, Kvell K, Talaber G, Veto S, Acs P, Gallyas F, et al. Thymic atrophy and apoptosis of CD4+CD8+ thymocytes in the cuprizone model of multiple sclerosis. *PLoS ONE.* (2015) 10:e0129217. doi: 10.1371/journal.pone.0129217
129. Martin NA, Molnar V, Szilagyi GT, Elkjaer ML, Nawrocki A, Okarmus J, et al. Experimental demyelination and axonal loss are reduced in MicroRNA-146a deficient mice. *Front Immunol.* (2018) 9:490. doi: 10.3389/fimmu.2018.00490
130. Herder V, Hansmann F, Stangel M, Schaudien D, Rohn K, Baumgartner W, et al. Cuprizone inhibits demyelinating leukomyelitis by reducing immune responses without virus exacerbation in an infectious model of multiple sclerosis. *J Neuroimmunol.* (2012) 244:84–93. doi: 10.1016/j.jneuroim.2012.01.010
131. Mana P, Fordham SA, Staykova MA, Correcha M, Silva D, Willenborg DO, et al. Demyelination caused by the copper chelator cuprizone halts T cell mediated autoimmune neuroinflammation. *J Neuroimmunol.* (2009) 210:13–21. doi: 10.1016/j.jneuroim.2009.02.013
132. Yakimov V, Schweiger F, Zhan J, Behrangi N, Horn A, Schmitz C, et al. Continuous cuprizone intoxication allows active experimental autoimmune encephalomyelitis induction in C57BL/6 mice. *Histochem Cell Biol.* (2019) 152:119–31. doi: 10.1007/s00418-019-01786-4
133. Partridge MA, Gopinath S, Myers SJ, Coorssen JR. An initial top-down proteomic analysis of the standard cuprizone mouse model of multiple sclerosis. *J Chem Biol.* (2016) 9:9–18. doi: 10.1007/s12154-015-0138-0
134. Komoly S, Jeyasingham MD, Pratt OE, Lantos PL. Decrease in oligodendrocyte carbonic anhydrase activity preceding myelin degeneration in cuprizone induced demyelination. *J Neurol Sci.* (1987) 79:141–8. doi: 10.1016/0022-510X(87)90268-1
135. Zatta P, Raso M, Zambenedetti P, Wittkowski W, Messori L, Piccioli F, et al. Copper and zinc dismetabolism in the mouse brain upon chronic cuprizone treatment. *Cell Mol Life Sci.* (2005) 62:1502–13. doi: 10.1007/s00018-005-5073-8
136. Moldovan N, Al-Ebraheem A, Lobo L, Park R, Farquharson MJ, Bock NA. Altered transition metal homeostasis in the cuprizone model of demyelination. *Neurotoxicol.* (2015) 48:1–8. doi: 10.1016/j.neuro.2015.02.009
137. Varga E, Pandur E, Abraham H, Horvath A, Acs P, Komoly S, et al. Cuprizone administration alters the iron metabolism in the mouse model of multiple sclerosis. *Cell Mol Neurobiol.* (2018) 38:1081–97. doi: 10.1007/s10571-018-0578-5
138. Venturini G. Enzymic activities and sodium, potassium and copper concentrations in mouse brain and liver after cuprizone treatment *in vivo*. *J Neurochem.* (1973) 21:1147–51. doi: 10.1111/j.1471-4159.1973.tb07569.x
139. Hopkins RG, Failla ML. Transcriptional regulation of interleukin-2 gene expression is impaired by copper deficiency in Jurkat human T lymphocytes. *J Nut.* (1999) 129:596–601. doi: 10.1093/jn/129.3.596
140. Bala S, Failla ML. Copper deficiency reversibly impairs DNA synthesis in activated T lymphocytes by limiting interleukin 2 activity. *Proc Natl Acad Sci USA.* (1992) 89:6794–7. doi: 10.1073/pnas.89.15.6794
141. Desdin-Mico G, Soto-Heredero G, Mittelbrunn M. Mitochondrial activity in T cells. *Mitochondrion.* (2018) 41:51–7. doi: 10.1016/j.mito.2017.10.006
142. Suzuki K. Giant hepatic mitochondria: production in mice fed with cuprizone. *Science.* (1969) 163:81–2. doi: 10.1126/science.163.3862.81
143. Suzuki K, Kikkawa Y. Status spongiosus of CNS and hepatic changes induced by cuprizone (biscyclohexanone oxalyldihydrazone). *Amn J Pathol.* (1969) 54:307–25.
144. Flatmark T, Kryvi H, Tangerås A. Induction of megamitochondria by cuprizone(biscyclohexanone oxaldihydrazone). Evidence for an inhibition of the mitochondrial division process. *Eur J Cell Biol.* (1980) 23:141–8.
145. Acs P, Komoly S. Selective ultrastructural vulnerability in the cuprizone-induced experimental demyelination. *Ideggyogyaszati Szemle.* (2012) 65:266–70.
146. Wakabayashi T. Megamitochondria formation - physiology and pathology. *J Cell Mol Med.* (2002) 6:497–538. doi: 10.1111/j.1582-4934.2002.tb00452.x
147. Taraboletti A, Walker T, Avila R, Huang H, Caporoso J, Manandhar E, et al. Cuprizone intoxication induces cell intrinsic alterations in oligodendrocyte metabolism independent of copper chelation. *Biochemistry.* (2017) 56:1518–28. doi: 10.1021/acs.biochem.6b01072
148. Shen Y, Wang X, Guo S, Qiu M, Hou G, Tan Z. Evolutionary genomics analysis of human nucleus-encoded mitochondrial genes: implications for the roles of energy production and metabolic pathways in the pathogenesis and pathophysiology of demyelinating diseases. *Neurosci Lett.* (2020) 715:134600. doi: 10.1016/j.neulet.2019.134600
149. Varhaug KN, Kråkenes T, Alme MN, Vedeler CA, Bindoff LA. Mitochondrial complex IV is lost in neurons in the cuprizone mouse model. *Mitochondrion.* (2020) 50:58–62. doi: 10.1016/j.mito.2019.09.003
150. Pasquini LA, Calatayud CA, Bertone Uña AL, Millet V, Pasquini JM, Soto EF. The neurotoxic effect of cuprizone on oligodendrocytes depends on the presence of pro-inflammatory cytokines secreted by microglia. *Neurochem Res.* (2007) 32:279–92. doi: 10.1007/s11064-006-9165-0
151. Guerineau M, Guerineau S, Gosse C. Abnormal mitochondrial dna molecules in megamitochondria from cuprizone-treated rats. *Eur J Biochem.* (1974) 47:313–9. doi: 10.1111/j.1432-1033.1974.tb03695.x
152. Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, et al. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Ann Neurol.* (2006) 59:478–89. doi: 10.1002/ana.20736
153. Senanayake VK, Jin W, Mochizuki A, Chitou B, Goodenowe DB. Metabolic dysfunctions in multiple sclerosis: implications as to causation, early detection, and treatment, a case control study. *BMC Neurol.* (2015) 15:154. doi: 10.1186/s12883-015-0411-4
154. Echaniz-Laguna A, Chassagne M, de Sèze J, Mohr M, Clerc-Renaud P, Tranchant C, et al. POLG1 variations presenting as multiple sclerosis. *Arch Neurol.* (2010) 67:1140–3. doi: 10.1001/archneurol.2010.219
155. Mao P, Reddy PH. Is multiple sclerosis a mitochondrial disease? *Bio Biophys Acta.* (2010) 1802:66–79. doi: 10.1016/j.bbadis.2009.07.002
156. Barcelos IP, Troxell RM, Graves JS. Mitochondrial dysfunction and multiple sclerosis. *Biology.* (2019) 8:37. doi: 10.3390/biology8020037
157. Sukumar M, Liu J, Mehta GU, Patel SJ, Roychoudhuri R, Crompton JG, et al. Mitochondrial membrane potential identifies cells with enhanced stemness for cellular therapy. *Cell Metabol.* (2016) 23:63–76. doi: 10.1016/j.cmet.2015.11.002
158. McTigue DM, Tripathi RB. The life, death, and replacement of oligodendrocytes in the adult CNS. *J Neurochem.* (2008) 107:1–19. doi: 10.1111/j.1471-4159.2008.05570.x
159. Gui J, Mustachio LM, Su DM, Craig RW. Thymus size and age-related thymic involution: early programming, sexual dimorphism, progenitors and stroma. *Aging Dis.* (2012) 3:280–90.
160. Hsu HC, Zhang HG, Li L, Yi N, Yang PA, Wu Q, et al. Age-related thymic involution in C57BL/6J × DBA/2J recombinant-inbred mice maps to mouse chromosomes 9 and 10. *Genes Immun.* (2003) 4:402–10. doi: 10.1038/sj.gene.6363982
161. Roden AC, Moser MT, Tri SD, Mercader M, Kuntz SM, Dong H, et al. Augmentation of T cell levels and responses induced by androgen deprivation. *J Immunol.* (2004) 173:6098–108. doi: 10.4049/jimmunol.173.10.6098
162. Tang S, Moore ML, Grayson JM, Dubey P. Increased CD8+ T-cell function following castration and immunization is countered by parallel expansion of regulatory T cells. *Cancer Res.* (2012) 72:1975–85. doi: 10.1158/0008-5472.CAN-11-2499

163. Harbo HF, Gold R, Tintoré M. Sex and gender issues in multiple sclerosis. *Therap Adv Neurol Dis.* (2013) 6:237–48. doi: 10.1177/1756285613488434
164. Booss J, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J Neurol Sci.* (1983) 62:219–32. doi: 10.1016/0022-510X(83)90201-0
165. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med.* (2000) 192:393–404. doi: 10.1084/jem.192.3.393
166. Witalison EE, Thompson PR, Hofseth LJ. Protein arginine deiminases and associated citrullination: physiological functions and diseases associated with dysregulation. *Curr Drug Target.* (2015) 16:700–10. doi: 10.2174/1389450116666150202160954
167. Raijmakers R, Vogelzangs J, Croxford JL, Wesseling P, van Venrooij WJ, Pruijn GJM. Citrullination of central nervous system proteins during the development of experimental autoimmune encephalomyelitis. *J Comp Neurol.* (2005) 486:243–53. doi: 10.1002/cne.20529
168. Yang L, Tan D, Piao H. Myelin basic protein citrullination in multiple sclerosis: a potential therapeutic target for the pathology. *Neurochem Res.* (2016) 41:1845–56. doi: 10.1007/s11064-016-1920-2
169. Scheld M, Rüther BJ, Große-Veldmann R, Ohl K, Tenbrock K, Dreymüller D, et al. Neurodegeneration triggers peripheral immune cell recruitment into the forebrain. *J Neurosci.* (2016) 36:1410–5. doi: 10.1523/JNEUROSCI.2456-15.2016
170. Baxi EG, DeBruin J, Tosi DM, Grishkan IV, Smith MD, Kirby LA, et al. Transfer of myelin-reactive th17 cells impairs endogenous remyelination in the central nervous system of cuprizone-fed mice. *J Neurosci.* (2015) 35:8626–39. doi: 10.1523/JNEUROSCI.3817-14.2015
171. Kirby L, Jin J, Cardona JG, Smith MD, Martin KA, Wang J, et al. Oligodendrocyte precursor cells present antigen and are cytotoxic targets in inflammatory demyelination. *Nat Com.* (2019) 10:3887. doi: 10.1038/s41467-019-11638-3
172. Zhou F, Lu M, Wang W, Bian ZP, Zhang JR, Zhu JJ. Electrochemical immunosensor for simultaneous detection of dual cardiac markers based on a poly(dimethylsiloxane)-gold nanoparticles composite microfluidic chip: a proof of principle. *Clinic Chemist.* (2010) 56:1701–7. doi: 10.1373/clinchem.2010.147256
173. Locatelli G, Wörtge S, Buch T, Ingold B, Frommer F, Sobottka B, et al. Primary oligodendrocyte death does not elicit anti-CNS immunity. *Nat Neurosci.* (2012) 15:543–50. doi: 10.1038/nn.3062
174. Oluich LJ, Stratton JA, Xing YL, Ng SW, Cate HS, Sah P, et al. Targeted ablation of oligodendrocytes induces axonal pathology independent of overt demyelination. *J Neurosci.* (2012) 32:8317–30. doi: 10.1523/JNEUROSCI.1053-12.2012
175. Traka M, Arasi K, Avila RL, Podojil JR, Christakos A, Miller SD, et al. A genetic mouse model of adult-onset, pervasive central nervous system demyelination with robust remyelination. *Brain.* (2010) 133:3017–29. doi: 10.1093/brain/awq247
176. Pohl HBF, Porcheri C, Mueggler T, Bachmann LC, Martino G, Riethmacher D, et al. Genetically induced adult oligodendrocyte cell death is associated with poor myelin clearance, reduced remyelination, and axonal damage. *J Neurosci.* (2011) 31:1069–80. doi: 10.1523/JNEUROSCI.5035-10.2011
177. Gritsch S, Lu J, Thilemann S, Wortge S, Mobius W, Bruttger J, et al. Oligodendrocyte ablation triggers central pain independently of innate or adaptive immune responses in mice. *Nat Com.* (2014) 5:5472. doi: 10.1038/ncomms6472
178. Gat-Viks I, Geiger T, Barbi M, Raini G, Elroy-Stein O. Proteomics-level analysis of myelin formation and regeneration in a mouse model for vanishing white matter disease. *J Neurochem.* (2015) 134:513–26. doi: 10.1111/jnc.13142
179. Oveland E, Nystad A, Berven F, Myhr KM, Torkildsen O, Wergeland S. 1,25-Dihydroxyvitamin-D3 induces brain proteomic changes in cuprizone mice during remyelination involving calcium proteins. *Neurochem Int.* (2018). 112:267–77. doi: 10.1016/j.neuint.2017.08.008
180. Carlton WW. Response of mice to the chelating agents sodium diethyldithiocarbamate, alpha-benzoinoxime, and biscyclohexanone oxaldihydrazone. *Toxicol App Pharmacol.* (1966) 8:512–21. doi: 10.1016/0041-008X(66)90062-7

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REVIEW
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Pre-clinical and Clinical Implications of "Inside-Out" vs. "Outside-In" Paradigms in Multiple Sclerosis Etiopathogenesis

Haley E. Titus[1†], Yanan Chen[2†], Joseph R. Podojil[1,3], Andrew P. Robinson[1], Roumen Balabanov[2], Brian Popko[2] and Stephen D. Miller[1,3,4]*

[1] Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, [2] Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, [3] Cour Pharmaceutical Development Company, Inc., Northbrook, IL, United States, [4] Interdepartmental Immunobiology Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States

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Antonio Luchicchi,
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***Correspondence:**
Stephen D. Miller
s-d-miller@northwestern.edu

[†] These authors have contributed equally to this work

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Multiple Sclerosis (MS) is an immune-mediated neurological disorder, characterized by central nervous system (CNS) inflammation, oligodendrocyte loss, demyelination, and axonal degeneration. Although autoimmunity, inflammatory demyelination and neurodegeneration underlie MS, the initiating event has yet to be clarified. Effective disease modifying therapies need to both regulate the immune system and promote restoration of neuronal function, including remyelination. The challenge in developing an effective long-lived therapy for MS requires that three disease-associated targets be addressed: (1) self-tolerance must be re-established to specifically inhibit the underlying myelin-directed autoimmune pathogenic mechanisms; (2) neurons must be protected from inflammatory injury and degeneration; (3) myelin repair must be engendered by stimulating oligodendrocyte progenitors to remyelinate CNS neuronal axons. The combined use of chronic and relapsing remitting experimental autoimmune encephalomyelitis (C-EAE, R-EAE) ("outside-in") as well as progressive diphtheria toxin A chain (*DTA)* and cuprizone autoimmune encephalitis (CAE) ("inside-out") mouse models allow for the investigation and specific targeting of all three of these MS-associated disease parameters. The "outside-in" EAE models initiated by myelin-specific autoreactive $CD4^+$ T cells allow for the evaluation of both myelin-specific tolerance in the absence or presence of neuroprotective and/or remyelinating agents. The "inside-out" mouse models of secondary inflammatory demyelination are triggered by toxin-induced oligodendrocyte loss or subtle myelin damage, which allows evaluation of novel therapeutics that could promote remyelination and neuroprotection in the CNS. Overall, utilizing these complementary pre-clinical MS models will open new avenues for developing therapeutic interventions, tackling MS from the "outside-in" and/or "inside-out".

Keywords: multiple sclerosis, etiopathogenesis, demyelination, autoimmunity, animal models

INTRODUCTION

Multiple Sclerosis presents most often in young adulthood and is chronic as most patients live with the disease for decades. Recent studies on prevalence uncovered that nearly a million people live with MS within the United States (Culpepper et al., 2019; Nelson et al., 2019; Wallin et al., 2019). Approximately 85% of patients present with the relapsing-remitting form of MS (RRMS) that involves episodes of neurological deficits followed by phases of recovery (Steinman, 2009). The disease often slowly converts to a secondary-progressive form of MS (SPMS) that shows significant and irreversible neurological impairment (Stadelmann, 2011). The primary-progressive form of MS (PPMS) appears in the remaining patients and results in rapid progressive neurological decline (Miller and Leary, 2007).

With advances in technology, in addition to the traditional family history portion of a patient's medical record, further characterization of a patient's predisposition to disease can be evaluated by genomic sequencing. For example, recent studies have identified over 200 genomic and proteomic anomalies prevalent in the MS patient population, all directly or indirectly linked to the immune system (International Multiple Sclerosis Genetics Consortium et al., 2013; Manconi et al., 2018; International Multiple Sclerosis Genetics Consortium, 2019; Kotelnikova et al., 2019). Clinical presentation of the disease as well as chronic neuropathology highlight the destructive nature of the interaction between the immune system and the CNS.

The "outside-in" hypothesis constitutes a primary pathogenesis of autoimmune inflammation followed by a secondary pathogenesis of myelin degradation (**Table 1**). The "inside-out" hypothesis is a primary pathogenesis of oligodendrocyte (OL) injury/myelin destabilization and a secondary pathogenesis due to activation of a reactive inflammatory response (**Table 1**). Experimental murine models can recapitulate patient clinical presentations and pathological changes including inflammatory demyelination, axonal pathology, and immune cell infiltration utilizing both "outside-in" immune mediated demyelination and the "inside-out" CNS demyelination/neurodegeneration driven models (**Table 2**).

Presently, there is no cure for MS and the long-term treatment of MS patients is based on disease-modifying therapies that either suppress or modulate the immune system, and symptomatic management. Ideally, the mechanism(s) of action for an efficacious therapy would function to specifically target the root cause of the immune and CNS dysfunction. First, the underlying autoimmunity must be mitigated through re-establishing self-tolerance (McCarthy et al., 2014; Luo et al., 2016; Pearson et al., 2017, 2019). Second, neurodegeneration must be mitigated to protect the remaining function of CNS neurons. Third, tissue repair within the CNS must restore oligodendrocyte insulation and myelin sheath formation around damaged axons (Rodgers et al., 2013). As the etiology of the disease is unknown, utilizing both "outside-in" and "inside-out" models for single selective immune regulatory and myelin repair therapy as well as combination therapy in pre-clinical trials are imperative for success in developing effective therapeutics in patient clinical trials. This review will focus on the "outside-in" models, "inside-out" models of MS, and the multi-directional feedback between the immune system and the CNS.

TABLE 1 | "Inside-out" and "Outside-in" hypotheses of MS pathophysiology.

	"Inside-Out" Hypothesis	"Outside-In" Hypothesis
Primary Pathogenesis	OL injury / Myelin destabilized	Autoimmune inflammation
Secondary Pathogenesis	Reactive inflammatory response/ Further myelin degradation	Myelin degradation

"OUTSIDE-IN" MS MODELS

The process of drug discovery, approval, and future patient use requires initial testing in experimental models that recapitulate hallmarks of the human disease state. Initial inflammatory demyelination in Multiple Sclerosis and subsequent neurodegeneration is a result of multi-directional feedback involving CNS resident cells (i.e., oligodendrocytes, neurons, and microglia) and infiltrating immune cells (i.e., autoreactive T cells and B cells, inflammatory monocytes, and macrophages) (McFarland and Martin, 2007; Bhat and Steinman, 2009; Weiner, 2009). While the primary etiology of MS remains unknown and is likely multi-determinant, the disease involves the activation of the peripheral adaptive immune system against CNS myelin epitopes. However, the triggering event that initiates this autoimmune response is not understood. Antigen presenting cells (APCs) (i.e., dendritic cells, monocytes, macrophages, microglia, and B cells) activate naïve $CD4^+$ T cells and promote differentiation of $CD4^+$ Th17 and Th1 cells through inflammatory cytokines (IL-1β, IL-6, and IL-23; and IL-12, respectively).

Activated microglia are rapidly recruited to sites of CNS damage (Duan et al., 2009). These APCs upregulate the expression of MHCII and other costimulatory molecules, such as CD40, CD80, and CD86 (Windhagen et al., 1995; Gerritse et al., 1996; Zrzavy et al., 2017). These observations suggest that activated APCs within the CNS possess the capacity to present antigens to infiltrating T cells. However, *in vitro* data show that microglia have a limited capacity to activate $CD4^+$ T cells (Mack et al., 2003). In contrast to the *in vitro* findings, a dynamic alteration in the presence and phenotype of microglia within the CNS has been reported with regard to the absence or presence of lesions within the local microenvironment (Esiri and Reading, 1987; Ferguson et al., 1997; Prineas et al., 2001; Zrzavy et al., 2017). For example, microglia activation was more pronounced and increased with the length of disease even in the normal-appearing white matter sections from MS patients as compared to control tissue samples (Zrzavy et al., 2017). Additionally, active lesions from MS patients contained microglia and macrophages expressing a pro-inflammatory phenotype (Zrzavy et al., 2017), suggesting the ability of these cells to activate $CD4^+$ T cells.

Besides microglia and macrophages, recent evidence suggests that B cells may serve as an important APC population in

TABLE 2 | "Inside-out" and "Outside-in" disease model systems highlighted in this review.

	"Inside-Out" models			"Outside-In" models			
	Epsilon Toxin Model	**Diphtheria Toxin A (DTA) Model**	**Cuprizone autoimmune encephalitis (CAE) Model**	**Chronic Experimental Autoimmune Encephalomyelitis (C-EAE) Model**	**Relapsing Remitting Experimental Autoimmune Encephalomyelitis (R-EAE) Model**	**Theiler's Virus-Induced Demyelinating Disease**	**Japanese Macaque Encephalomyelitis (JME) Model**
Model species	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Macaque
Induction of disease model (in Adults)	Epsilon toxin, produced by type B and D strains of *Clostridium perfringens*, a spore-forming gram-positive bacterium	Timed genetic expression of diphtheria toxin (tamoxifen induced *PLPCreER*^{T} for targeting OL)	Cuprizone diet for 2 weeks, then inject CFA (SubQ) and Pertussis Toxin (IP)	MOG_{35-55} + CFA (SubQ) and Pertussis Toxin (IP)	$PLP_{139-151}$ + CFA (SubQ)	Theiler's Murine Encephalomyelitis Virus (TMEV) intracerebral infection	Japanese Macaque Rhadinovirus (JMRV), spontaneous or injected
Disease model trigger	Epsilon Toxin induced cytotoxicity (OL)	DTA induced cell death (OL), secondary MOG peptide immune response	Cuprizone destabilizes myelin, Citrullinated MBP drives immune response	MOG_{35-55} peptide immune response	$PLP_{139-151}$ peptide immune response	Response to TMEV and subsequent spreading to PLP and MBP epitopes	JMRV infection, MBP peptide immune response
Disease model pathogenesis	OL cytotoxicity, triggers demyelination	OL ablation, triggers demyelination/ remyelination, secondary immune response (respond to MOG peptide)	Myelin breakdown, secondary immune response (respond to MBP epitope)	Immune infiltration (respond to MOG peptide), secondary CNS degeneration	Immune infiltration (initially respond to PLP peptide, later to MBP), secondary CNS degeneration	Immune response to virus, release of myelin epitopes inducing autoimmune pathology, secondary CNS degeneration	Immune response to virus and infiltration (respond to MBP peptide), secondary CNS degeneration

RRMS disease pathogenesis. To test this hypothesis, the ability of memory B cells from RRMS patients to activate $CD4^+$ T cell in response to MBP and MOG was compared to naïve B cells from health donors. The data show that the naïve B cells from healthy donors did not activate the $CD4^+$ T cells in the presence of MBP and MOG, while the memory B cells from RRMS patients did activate the $CD4^+$ T cells (Harp et al., 2010). In the context of anti-CD20 therapy, which deletes peripheral B cells, the aforementioned findings suggest that the depletion of B cells following anti-CD20 treatment may be due in-part to the loss of B cells as an APC population. This possibility is supported by studies utilizing whole MOG protein-induced EAE in C57BL/6 mice, in which MOG-specific B cells are activated (Hausler et al., 2018).

In the "inside-out" model of MS (**Figure 1**), amplified inflammation, driven by peripherally derived autoreactive $CD4^+$ Th17 and Th1 cells, directly and indirectly leads to further myelin destruction (Glass et al., 2010; Prinz and Kalinke, 2010). Based on the presence of T cell-mediated inflammation within the CNS of MS patients, the field historically utilizes "outside-in" experimental models of disease (**Figure 2**). These experimental models include relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE) and Theiler's murine encephalomyelitis virus (TMEV) infection in SJL/J mice, chronic experimental autoimmune encephalomyelitis (C-EAE) in C57BL/6 mice, and more recently a non-human primate model of virus-induced Japanese macaque encephalomyelitis (JME) (**Table 2**).

Experimental Autoimmune Encephalomyelitis (EAE)

In mice, the MS disease processes, including myelination defects, axonal pathology, and immune cell infiltration can be experimentally recapitulated. Classically, the experimental autoimmune encephalomyelitis (EAE) mouse model has been used to mimic autoimmune demyelination in response to a peripheral immune-priming event serving as an "outside-in" approach. Both relapsing-remitting MS (RR-MS) and primary-progressive MS (PP-MS) can be arguably modeled by EAE induced via subcutaneous priming of different mouse strains with specific myelin peptides in complete Freund's adjuvant (CFA). $CD4^+$ Th1/17 cells primed in the peripheral lymph nodes, traffic to the CNS, and are re-stimulated with endogenous myelin antigens leading to effector responses and clinical disease. Priming SJL/J mice with proteolipid protein $(PLP)_{139-151}$/CFA results in multiple clinical relapses (R-EAE) and priming C57BL/6 mice with myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$/CFA and pertussis toxin or infection of SJL/mice with TMEV induces an acute phase of disease followed by chronic progression (C-EAE) as measured by clinical scoring (Theiler and Gard, 1940; Veillette et al., 1989; Ben-Nun et al., 1991; Burns et al., 1991; Sun et al., 1991; Trotter et al., 1991; Soderstrom et al., 1993; Zhang et al., 1993; Steinman et al., 2002; Sospedra and Martin, 2005; Robinson et al., 2014; Terry et al., 2016). The use of the R-EAE and C-EAE mouse models allow assessment of motor function via clinical scoring, immune cell function,

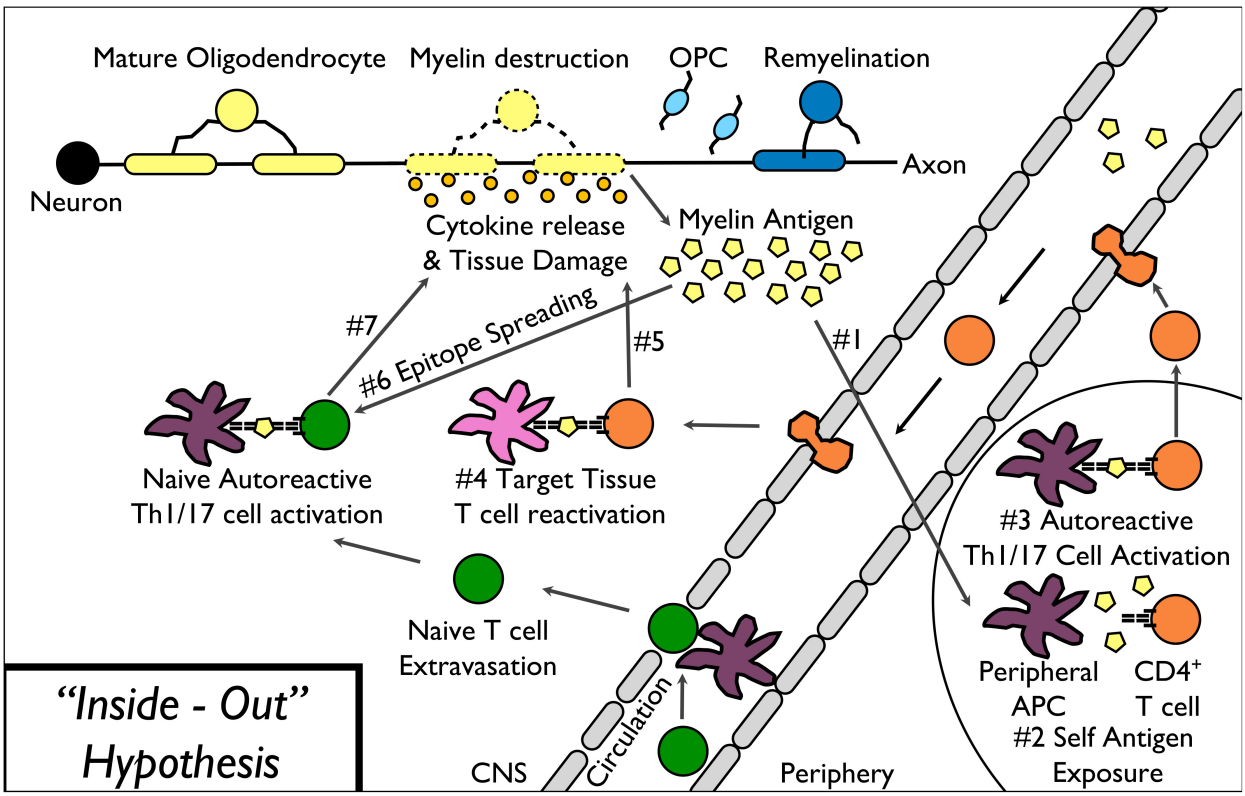

FIGURE 1 | "Inside-Out" model of MS pathophysiology. The "inside-out" model of MS pathogenesis begins with the release of myelin antigens from injured or destabilized myelin to the periphery (1) followed by the presentation of myelin epitopes to (2) and activation of autoreactive T cells (3). Activated autoreactive T cells then migrate into the CNS, are reactivated by CNS-resident APCs (4), and release cytokines leading to direct as well as indirect damage to myelin (5). Additional myelin epitopes released by the primary T cell response induce epitope spreading (6) leading to additional myelin destruction (7).

promotion of oligodendrocyte proliferation/maturation, and formation of new myelin.

Ideally, re-establishing self-tolerance would be induced by antigen-specific immune therapy (i.e. immune tolerance) in which the remaining immune system functions remain intact. The utilization of both R-EAE and C-EAE models of disease have been used to identify the underlying epitope-spreading mechanism within autoimmune disease. Epitope spreading in R-EAE has been clearly defined during the various phases of disease (Miller et al., 1995). During the immune response to a foreign or self-protein, the initial $CD4^+$ T cell response focuses on one or two antigenic peptide epitopes within the immunogenic protein. These initial immunogenic epitope(s) are termed the dominant epitope(s). As the immune response progresses, the process of epitope spreading occurs, which is defined as the activation of additional antigen-specific $CD4^+$ T cells that express T cell receptors specific for additional antigens that are not the dominant epitope(s) (Lehmann et al., 1992, 1993; Vanderlugt and Miller, 2002). For example, in an SJL/J mouse primed with $PLP_{139-151}$/CFA, $PLP_{139-151}$-specific $CD4^+$ T cell reactivity is induced within 3 days of priming in the draining lymph nodes for the site of $PLP_{139-151}$/CFA injection, and this dominant epitope-specific $CD4^+$ T cell response is maintained throughout the disease course. Immediately before, and continuing during, the primary relapse phase of disease, $PLP_{178-191}$ reactivity (termed intramolecular epitope spreading, i.e., spreading from one peptide epitope to another peptide epitope contained within the same protein) is detected by T cell proliferation and delayed-type hyper-sensitivity (DTH) assays. During the secondary relapse phase of disease, MBP_{84-104} responses (termed intermolecular epitope spreading, i.e., spreading from one peptide epitope to another peptide epitope contained within a different protein) are detectible. Conversely, if SJL/J mice are primed with $PLP_{178-191}$/CFA, the acute phase of disease is mediated by $CD4^+$ T cell responses to the initiating $PLP_{178-191}$ epitope. Subsequently, $PLP_{139-151}$ $CD4^+$ T cells are detectible within the spleen and cervical lymph nodes during the primary disease relapse, and MBP_{84-104} specific $CD4^+$ T cells during the secondary disease relapse. Published data show that while the detection of the spread epitope-specific $CD4^+$ T cells ($PLP_{178-191}$ or $PLP_{139-151}$ specific $CD4^+$ T cells depending on the peptide used to induce disease) does not occur until the primary disease relapse, these spread epitope-specific $CD4^+$ T cells are initially activated during the acute phase of disease via antigen presenting cells presenting spread epitope peptides within the CNS (McMahon et al., 2005). Similarly, infection of SJL/J mice with TMEV, results in the bystander immune-mediated CNS damage leading to initial epitope spreading to $PLP_{139-151}$ followed by responses to additional myelin epitopes. The development of these responses

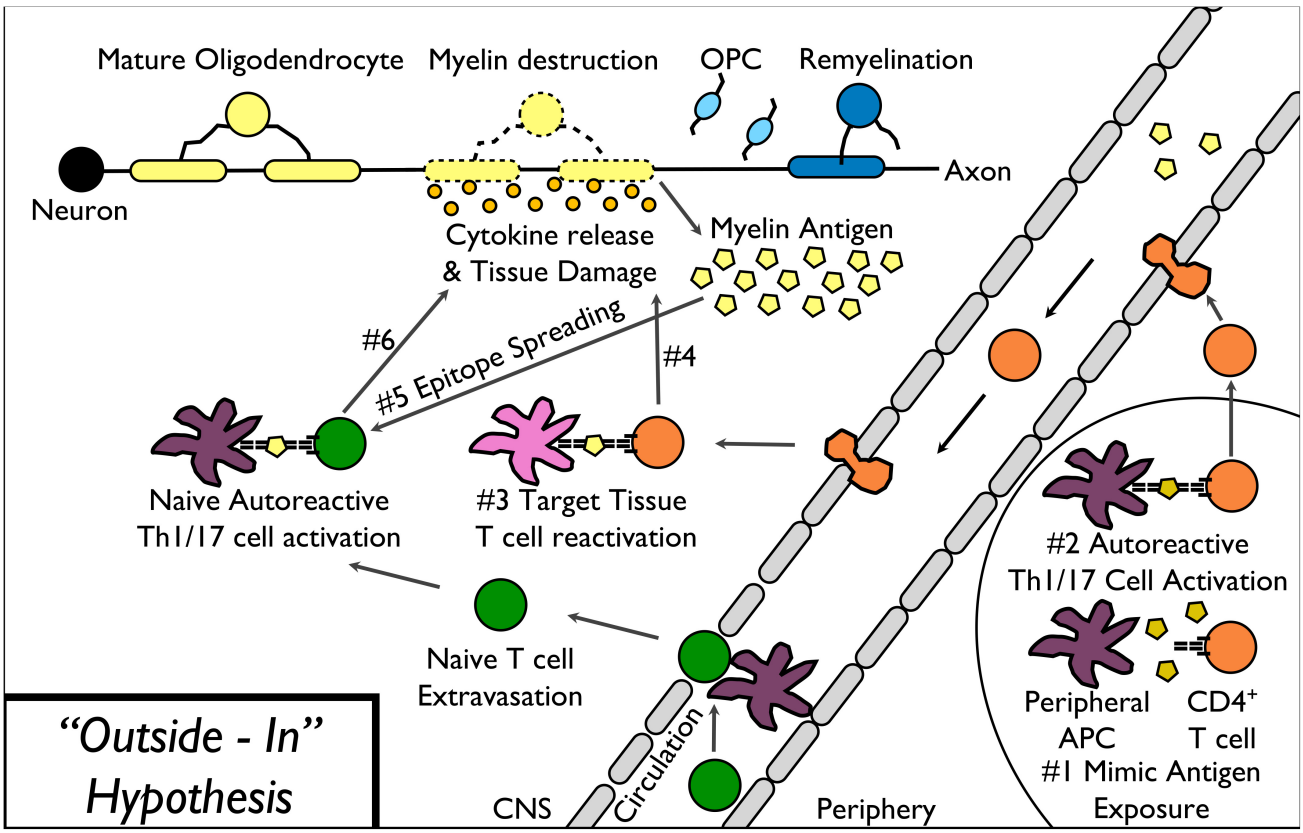

FIGURE 2 | "Outside-In" model of MS pathophysiology. The "outside-in" model of MS pathogenesis begins with activation of myelin-specific T cells in response to a myelin peptide mimic epitope expressed on a pathogenic virus or other microbe exposure (1–2). Activated autoreactive T cells then migrate into the CNS, are reactivated by CNS-resident APCs (3), and release cytokines leading to direct as well as indirect damage to myelin (4). Additional myelin epitopes released by the primary T cell response induce epitope spreading (5) leading to additional myelin destruction (6).

correlates with the extent of myelin destruction during the acute disease phase (McRae et al., 1995). The hierarchy of dominant epitopes is due to a combination of differential protein processing and presentation by various APCs, and the precursor frequency of the antigen-specific $CD4^+$ T cells (Lehmann et al., 1998; Moon et al., 2007).

The epitope spreading phenomena during autoimmune disease has been confirmed by the use of antigen-specific tolerance therapies. For example, immune tolerance is readily induced by coupling of peptides to donor splenocytes (SP) using the chemical crosslinker 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (ECDI) (Wetzig et al., 1979). Antigen coupled to splenocytes (Ag-SP) delivers the antigen to APCs that present the cargo antigen in a tolerogenic manner. The non-specific crosslinking of antigen to the cell surface while inducing apoptosis allows the donor cells to be perceived by the host in a non-inflammatory (non-immunogenic) manner. Ag-SP have been employed to prevent and treat the relapsing EAE model of MS (Podojil and Miller, 2009), and type 1 diabetes (T1D) in the non-obese diabetic (NOD) mouse (Prasad et al., 2012). A recent publication summarized the results of a phase I trial in MS patients using apoptotic ECDI-fixed peripheral blood mononuclear cells (PBMCs) pulsed with a cocktail of myelin peptides, illustrating the safety and efficacy of this procedure in human autoimmune disease (Lutterotti et al., 2013). Importantly, the mechanistic aspects of this study provided an important proof-of-principle that induced peripheral tolerance can be successfully employed to induce unresponsiveness in human autoreactive T cells.

More recently, biodegradable carboxylated nanoparticles composed of poly(lactic-*co*-glycolic) acid (PLGA) were shown to induce antigen-specific tolerance for prevention and treatment of EAE when encephalitogenic peptides were EDCI fixed to the surface of the particles or encapsulated within the particles (Getts et al., 2012; Hunter et al., 2014). Administration of Ag-bearing PLGA nanoparticles results in significantly reduced CNS infiltration of encephalitogenic Th1 (IFN-γ) and Th17 (IL-17a and GM-CSF) cells as well as inflammatory monocytes/MΦs. Tolerance was most effectively induced by intravenous infusion of Ag-PLG (Getts et al., 2012), though intraperitoneal delivery was also able to attenuate disease scores. The intravenous route likely has greater efficacy due to direct trafficking and uptake of the nanoparticles by APCs in the liver and spleen via the macrophage receptor of collagenous structure (MARCO) scavenger receptor (Getts et al., 2011).

Theiler's Murine Encephalomyelitis Virus-Induced Demyelinating Disease (TMEV-IDD)

As outlined above, we have previously extensively studied and reviewed (Croxford et al., 2002; Munz et al., 2009) the immunopathogenesis of TMEV-induced demyelinating disease (TMEV-IDD) "outside-in" model of MS. Briefly, TMEV is a picornavirus which naturally enters the CNS via a fecal-oral transmission route and enters the CNS via a retrograde transport mechanism. In experimental TMEV-IDD, disease is induced by intracerebral injection of TMEV which then induces a persist infection of microglia which, in susceptible mouse strains, stimulates inflammatory anti-viral immune responses (Th1, Th17, and CD8) which cause bystander damage to oligodendrocytes and myelin in the CNS. Released myelin antigens then activate myelin epitope-specific autoimmune responses via the process of epitope spreading (Miller et al., 1997) leading to a chronic demyelinating and a spastic course of paralysis. We also showed that a strain of TMEV engineered to express molecular mimic of the myelin $PLP_{139-151}$ epitope sharing only 3 of the 13 amino acid residues (critically including the primary MHC binding and the primary and secondary T cell receptor binding residues), could induce demyelinating disease via the process of molecular mimicry (Olson et al., 2001). Collectively these studies indicate that myelin-specific autoimmune pathology can be induced by infection both via bystander damage induced release of self-antigens (epitope spreading) and molecular mimicry.

Japanese Macaque Encephalomyelitis (JME)

Japanese macaque encephalomyelitis (JME) is an inflammatory demyelinating disease that occurs spontaneously in a colony of Japanese macaques (JM) at the Oregon National Primate Research Center (Axthelm et al., 2011; Blair et al., 2016). The disease only occurs in specific lineages within the colony, and is triggered by a novel gamma-herpes virus, Japanese macaque rhadinovirus (JMRV), that occurs spontaneously in 1–3% of the JM colony and with targeted breeding 2–5% (Axthelm et al., 2011). If needed, based on population and time constraints, disease can be induced by intracranial injections of JMRV into animals from affected lineages (Estep et al., 2013) with the advantage of a known consistent location for histology and MRI/DTI. Animals with JME display clinical signs resembling Multiple Sclerosis, such as; ataxia, paresis, and magnetic resonance imaging reveals multiple T2-weighted hyperintensities and gadolinium-enhancing lesions in the central nervous system (i.e., brainstem, cerebellum, and cervical spinal cord). The prevalent myelin epitope is myelin basic protein (MBP). Comparable to disease manifestation in MS patients, the CNS of animals with JME present with active lesions that contain $CD4^+$ Th1 and Th17 cells, $CD8^+$ T cells, and oligoclonal bands are present within the CSF (Blair et al., 2016).

In addition to testing immune modulatory therapies, therapies that potentially promote myelin repair by stimulating oligodendrocyte progenitor cell expansion, homing and/or differentiation can be assessed in the EAE and TMEV-IDD mouse and JME primate models of MS. Researchers can examine clinical disease progression in the form of sensory and motor function, CNS immune and inflammatory responses, flow cytometry-based enumeration of cells of the oligodendrocyte lineage, and changes in myelin. The use of the MS-like animal disease models provides a robust platform for assessing combined immune regulation and myelin repair therapies in an "outside-in" model of CNS immune-induced demyelinating disease. These *in vivo* platforms for testing new myelin repair drugs will hopefully lead to the translation of a novel drug, either alone or in combination with immune regulatory drugs, for the treatment of MS.

"INSIDE-OUT" MS MODELS

The "inside-out" model proposed by Stys et al. (2012) argues that primary degeneration of oligodendrocytes and myelin is the initial event of MS, and might occur in the earliest years before the onset of symptoms. Primary oligodendrocyte death and/or subtle myelinopathy can precede and subsequently drive a secondary autoimmune attack, resulting in inflammatory demyelination in MS (**Figure 1**). Therefore, there has been a search for agents that could trigger these CNS events, resulting in the onset of an immune response to myelin.

Epsilon Toxin Model

In the earliest stage of MS, the histologic description of formation of nascent lesions without inflammatory infiltration argues for the possibility of an "inside-out" mechanism (Barnett and Prineas, 2004; Prineas and Parratt, 2012). Observation of oligodendrocyte apoptosis along with blood brain barrier disruption in the nascent lesions indicates that MS might arise from an environmental insult targeting oligodendrocytes, such as a toxin or virus. Epsilon toxin is produced by the type B and the type D strains of *Clostridium perfringens*, a spore-forming gram-positive bacterium mostly found in the intestines of ruminant animals (Blackwell et al., 1983; Uzal et al., 2004; Uzal and Songer, 2008). Epsilon toxin is converted into an active form, crosses the intestinal mucosa and disseminates via the bloodstream, massively accumulating in the brain and kidneys (Finnie, 1984, 2003; Tamai et al., 2003). The toxin has the capability to cross the blood brain barrier and infiltrate the brain parenchyma, which results in MS-like symptoms (Dorca-Arevalo et al., 2008; Popoff, 2011). Over three decades ago, Murrell et al. (1986) first hypothesized that epsilon toxin is the potential toxin that triggers MS, even though humans are not natural hosts for *C. perfringens* types B and D. More recently, *C. perfringens* type B was isolated from the stool of a female remitting-relapsing MS patient with an onset 3 months previously (Rumah et al., 2013). Furthermore, epsilon toxin specific antibodies were found in serum and/or CSF of 10% of MS patients and 1% of healthy controls from the banked samples in the United States (Rumah et al., 2013). Similarly, immunoreactivity toward epsilon toxin in serum is higher in MS patients than in controls in the United Kingdom (Wagley et al., 2019). In light of these observations and clinical evidence, it has

been proposed that epsilon toxin exposure may play a role in initiating MS lesion formation by binding to oligodendrocytes, myelin and white matter (Lonchamp et al., 2010; Wioland et al., 2015). Although the mechanism underlying the effect of epsilon toxin on oligodendrocytes and subsequent demyelination is not yet clear, several lines of evidence *in vitro* indicate that epsilon toxin selectively attacks mature oligodendrocytes and triggers demyelination (Linden et al., 2015; Wioland et al., 2015; Bossu et al., 2020). It has been shown that myelin and lymphocyte protein (MAL) could be a candidate epsilon toxin receptor on oligodendrocytes (Rumah et al., 2015). Once bound to oligodendrocytes, epsilon toxin could lead to the rise of glutamate and subsequent activation of mGluR, which activates intracellular Ca^{2+}signaling and eventually triggers demyelination (Lonchamp et al., 2010; Kostic et al., 2013; Wioland et al., 2015). In line with this evidence, it is highly possible that an agent cytotoxic to oligodendrocytes may trigger MS.

Diphtheria Toxin A Chain (DTA) Model

One of the proposed factors in the alternative "inside-out" theory for initiating MS is the primary cytotoxic death of oligodendrocytes. A toxin-induced ablation of oligodendrocytes is useful for testing whether such oligodendrocyte death could trigger anti-CNS autoimmunity (Jakel and Dimou, 2017). *Plp1-CreER*^{T}*; ROSA26-eGFP-DTA* (DTA) is a mouse model of oligodendrocyte ablation accomplished via an oligodendrocyte specific activation of toxin expression in adult mice. The A subunit of diphtheria toxin (*DT-A*) induces cell death by catalyzing the inactivation of elongation factor 2, thereby halting global protein synthesis (Collier, 2001). The *Plp1-CreER*^{T} mouse line drives expression of the tamoxifen-regulated Cre recombinase under control of the oligodendrocyte-specific myelin proteolipid protein (PLP) transcriptional control region (Doerflinger et al., 2003). The *DT-A* expression in oligodendrocytes is the result of tamoxifen-induced Cre-mediated recombination of *ROSA26-eGFP-DTA* locus via *Plp1-CreER*^{T} (Traka et al., 2010). The expression of the *DT-A* subunit specifically in CNS myelinating oligodendrocytes results in widespread oligodendrocyte ablation, CNS demyelination, and the subsequent development of severe neurological symptoms. There is no breakdown of the blood brain barrier or detectible increase in $CD4^+$ T cell into the CNS despite local inflammation, and there is no apparent loss of CNS axons during the initial demyelination event. However, the specific phenotype(s) of the T cells within the CNS during this initial demyelination phase of disease have not been fully characterized. The clinical symptoms of the acute phase of the disease are ameliorated during a recovery phase that correlates with the repopulation of mature oligodendrocytes and robust remyelination in the following 6–7 weeks (Traka et al., 2010). Interestingly, as the recovered mice age, they develop a secondary lethal progressive demyelinating disease starting around 40 weeks after tamoxifen injection that is mediated by MOG_{35-55}-specific $CD4^+$ T cell infiltration into the CNS during the late stages of disease (Traka et al., 2016). While the MOG_{35-55}-specific $CD4^+$ T cell responses are detectable in the peripheral lymphoid organs at 40 weeks post induction and are not present at 10 weeks, there is a significant increased number of $CD4^+$ T cells in the CNS at 10 weeks (Traka et al., 2016). This increase in CNS $CD4^+$ T cells may correlate with the expansion of myelin-specific T cells, similar to the initial activation and expansion of spread-epitope-specific $CD4^+$ T cells within the CNS in EAE (McMahon et al., 2005; Bailey et al., 2007). The late-stage pathology is due to the induction of $CD4^+$ T cell-mediated autoimmune responses secondary to oligodendrocyte ablation via a non-immune-mediated event (i.e. DT-mediated toxicitiy). This is supported by the major findings that adoptive transfer of the myelin-specific $CD4^+$ T cells derived from DTA mice into naïve mice consistently results in the induction of mild neurological symptoms and inflammatory CNS lesions in the recipients, and induction of immune tolerance using the MOG_{35-55}-coupled PLG nanoparticles significantly inhibits the progression of late-onset disease symptoms in DTA mice protecting animals from eventual fatal demyelinating disease (Traka et al., 2016).

In addition to the DTA mouse model, other genetic mouse models were later developed to achieve a faster oligodendrocyte ablation via expressing diphtheria-toxin receptor (DTR) under the MOG-promoter accompanied by direct administration of diphtheria toxin (Ghosh et al., 2011; Locatelli et al., 2012; Oluich et al., 2012; Gritsch et al., 2014). However, some studies reported that a secondary anti-CNS immunity did not develop in these mice, that is most likely due to premature death of these mice (Locatelli et al., 2012; Gritsch et al., 2014). The development of CNS immunity after oligodendrocyte death appears to be a slow process, taking several months in the DTA mouse model described above.

The DTA mouse model supports the "inside-out" theory, recapitulating pathological evidence showing that the loss of oligodendrocytes and subsequent demyelination may result in the induction of autoreactivity against myelin antigens as well as secondarily lead to inflammation and demyelination in the CNS. The unique DTA mouse model system allows fundamental unanswered questions concerning the molecular and cellular mechanisms associated with the induction of the autoimmune response to contribute to the understanding of MS disease pathogenesis and to the development and testing of remyelination therapies.

Cuprizone Autoimmune Encephalitis (CAE) Model

In addition to diphtheria-toxin, cuprizone is a demyelinating neurotoxin that has been used in testing "inside-out" hypothesis. Long-term of cuprizone feeding in mice lead to oligodendrocyte death, demyelination and gliosis (Matsushima and Morell, 2001; Sen et al., 2019b). Unlike DTA model, cuprizone feeding did not evoke a peripheral immune response in the CNS (Caprariello et al., 2018; Sen et al., 2019a). Some studies reported that the failure of cuprizone feeding to trigger in triggering CNS immune response is due to the atrophy of immune organs like the spleen and thymus (Solti et al., 2015; Partridge et al., 2016; Sen et al., 2019a). A more recent study reported that cuprizone induced demyelination can trigger an

"inside-out" immune response when the BBB is disrupted by pertussis toxin (Almuslehi et al., 2020).

Accumulating clinical evidence suggests that primary myelin destabilization by citrullination releases immunogenic myelin debris and subsequently drives a secondary autoimmune attack (Moscarello et al., 1994; Cao et al., 1999; Stys et al., 2012). Excessive citrullination of myelin basic protein (MBP) had been found in normal appearing white matter from postmortem MS brain tissues and the extent of modified myelin is related to the severity of MS (Moscarello et al., 1994; Wood et al., 1996; Bradford et al., 2014). Citrullination is a post-translational modification mediated by peptidylarginine deiminase (PAD). Citrullination occurs when a positively charged arginine residue is deiminated to a neutrally charged citrulline (Vossenaar et al., 2003; Moscarello et al., 2007). Due to the changed charge in the protein, citrullinated MBP is partially unfolded and cannot stabilize a compact myelin sheath (Beniac et al., 2000; Bakhti et al., 2013). Studies have shown that deiminated MBP with citrulline is more susceptible to proteolytic digestion and that the digestion rate is remarkably correlated with the amount of citrulline present in MBP peptides (Cao et al., 1999; D'Souza and Moscarello, 2006; Musse et al., 2006). Increased breakdown of citrullinated MBP results in generating immunodominant epitopes (Musse et al., 2006); potentially triggering autoimmunity and eliciting destructive inflammatory demyelination (Raijmakers et al., 2005).

A newly developed mouse model of cuprizone autoimmune encephalitis (CAE) provides direct evidence to support the causative relationship between primary abnormalities of myelin and inflammation (Caprariello et al., 2018), whereby biochemical destabilizing myelin triggers a secondary inflammatory demyelination comparable to active MS lesions. The CAE is initiated with a 2-week exposure of neurotoxicant cuprizone to perturb myelin without causing overt demyelination, followed by an immune boost of complete Freund's adjuvant (without exogenous antigen) and pertussis toxin. After 2 weeks, these mice develop inflammatory demyelination which resembles pathology found in MS patients and the EAE mouse model (Caprariello et al., 2018). The histopathological changes of the CAE model are characterized by periventricular and white matter tract gadolinium enhancement of MRI of the brain as well as overt demyelination and cellular infiltration within the corpus callosum. Gadolinium enhancement indicates breakdown of the BBB as a result of active inflammation. However, removal of the immune boost abrogates these responses, implying the importance of an immune-permissive environment. Most importantly, suppression of the destructive immune response by administration of peptidyl arginine deiminase (PAD) inhibitors to the CAE mice suggests that citrullinated proteins altered by abbreviated cuprizone exposure possibly drive the inflammatory demyelinated lesions in CAE.

Additional "Inside-Out" Models

Although genetic mutations of myelin proteins as well as traumatic brain injury-associated dysmyelination have been linked to later development of MS, it is not yet clear what initially triggers citrullination of myelin proteins or subtle dysmyelination (Warshawsky et al., 2005; Donovan et al., 2014; Sidaway, 2017; Cloake et al., 2018).

Adrenoleukodystrophy (ALD) is an X-linked neurometabolic disorder due to mutations in a proximal transporter, adenosine triphosphate (ATP)-binding cassette, subfamily, member 1 gene (*ABCD1*) (Moser et al., 2007; Kemp et al., 2016). The clinical presentation of ALD is complex; involving adrenal insufficiency and myelopathy (de Beer et al., 2014; Kemp et al., 2016). Approximately 60% of male patients develop rapidly progressive inflammatory cerebral demyelination (Moser et al., 1992), which clinically coincides with a progressive neurological decline similar to MS (Ferrer et al., 2010; Brandao de Paiva et al., 2018). However, the complex mechanisms on how this metabolic disease is transitioned to a fatal neuroinflammatory disease remains elusive. *ABCD1* mutation may prevent transport of very long-chain fatty acids (VLCFAs) into peroxisomes for oxidation and degradation (Moser et al., 2007; Kemp et al., 2016). Some studies suggest that the accumulation of VLCFAs in myelin could mediate myelin instability and initial demyelination, which are believed to contribute to initiation of the inflammatory disease (Ho et al., 1995; Ito et al., 2001; Singh et al., 2009). The findings of CD1 (antigen presenting molecule) -mediated lipid antigen presentation in cerebral ALD lesions supported the hypothesis that VLCFA-containing proteolipid protein in myelin may be a potential lipid antigen for triggering autoimmunity after myelin breakdown (Ito et al., 2001). The lesions progress rapidly accompanied by the opening of blood brain barrier and invasions of inflammatory cells (Powers et al., 1992; Ito et al., 2001). Further evidence for the involvement of different components of the immune system in the pathogenesis of demyelinating ALD was reviewed in Hudspeth and Raymond (2007). Interestingly, the demyelinating progress arrested in a small percentage of patients with initial cerebral demyelination, in which the disruption of the blood brain barrier does not occur (Korenke et al., 1996). The importance of blood brain barrier in ALD was emphasized by several pieces of evidence that suggested potential environmental factors, such as head trauma (Weller et al., 1992; Raymond et al., 2010), possibly increase permeability of the blood brain barrier, which either trigger or precipitate the demyelination.

Traumatic brain injury (TBI) is most commonly caused by an external head impact that injures the brain. Demyelination and irreversible axon damage, particular in the corpus callosum, represent major pathological features frequently observed in TBI patients (Rutgers et al., 2008; Armstrong et al., 2016a; Chung et al., 2018; O'Phelan et al., 2018). Nevertheless, the progression of white matter injury is poorly understood in TBI. Oligodendrocytes are known to be vulnerable to oxidative stress and excitotoxicity following traumatic injury (Lotocki et al., 2011; Giacci and Fitzgerald, 2018). The loss of oligodendrocytes could significantly contribute to underlying demyelination after injury (Dent et al., 2015; Armstrong et al., 2016b) and activate neuroinflammation (Mierzwa et al., 2015). Furthermore, the observation that a persistent adaptive immune response in the CNS developed in the mice weeks after TBI (Daglas et al., 2019) fits within the "inside-out" theory. Interestingly, neuroinflammation, which persists for years after TBI, has recently been shown to largely contribute

to neurodegeneration and long-term neurological dysfunction (Bazarian et al., 2009; Amor et al., 2010; Daglas et al., 2019). In particular, genetic depletion of CD8+ T cells in TBI mouse model improves neurological outcomes (Daglas et al., 2019), which indicates the importance of neuroinflammation in the progression of TBI. Encouragingly, damping neuroinflammation with immunomodulatory nanoparticles results in reduced neuropathology and neurophysiological abnormalities following TBI, suggesting a potential therapeutic strategy (Sharma et al., 2020).

Pre-clinical and clinical findings in ALD as well as TBI could shed light on potential MS therapeutics and vice versa. Current MS treatments are mainly directed to immune suppression, but the CAE model provides evidence for a potential "inside-out" mechanism of initiation of chronic demyelination and could serve as a compelling preclinical model of MS translational studies for the development of myelin-protective strategies in early stages of the disease.

RELEVANCE FOR THE CLINIC

As we have highlighted the "outside-in" (**Figure 2**) peripheral immune driven models and "inside-out" (**Figure 1**) neurodegenerative models (**Table 2**), the next section will focus on the relevance for the clinic. The "outside-in" pathogenesis (**Figure 2**) begins with activation of myelin-specific T cells in response to a myelin peptide mimic epitope expressed on a pathogenic virus or other microbe exposure. Activated autoreactive T cells then migrate into the CNS, are reactivated by CNS-resident APCs, and release cytokines leading to direct as well as indirect damage to myelin. Additional myelin epitopes released by the primary T cell response induce epitope spreading, leading to additional myelin destruction. During repair, a mild inflammatory reaction can stimulate oligodendrocyte precursor cells and "protective autoimmunity" utilizing T regulatory cells (Schwartz and Raposo, 2014). Unfortunately, the eventual failure of myelin repair during RRMS leads to chronically demyelinated axons, which degenerate over time and contribute to disease progression (Franklin and Ffrench-Constant, 2008; Trapp and Nave, 2008). Neuronal injury occurs, in part, as a result of inflammation mediated by myelin-specific $CD4^+$ T cells. Direct and indirect effects of neuroantigen-specific Th1/17 cells can lead to demyelination and subsequent neuronal dysfunction by mechanisms that include activation of microglia and infiltrating inflammatory monocytes/macrophages by pro-inflammatory cytokines (IFN-γ, IL-17, and GM-CSF), which then produce proteases and additional pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS), which induce myelin and axonal damage (Glass et al., 2010). Neuronal loss is thought to be a consequence of the demyelination, which causes dramatic ionic and energy imbalances in axons resulting from the loss of the structural and trophic support provided by oligodendrocytes (Trapp and Nave, 2008; Nave, 2010). This subsequent "inside-out" pathogenesis (**Figure 1**) can then spread and lead to further loss of axonal integrity. This perpetuates the release of myelin antigens to the periphery followed by the presentation of myelin epitopes to and activation of autoreactive T cells; ultimately leading to the progressive diffuse atrophy of the brain.

MS Patient Therapeutics

Disease modifying therapies for MS include immunomodulatory and immunosuppressive medications that suppress or modulate the self-reactive immune responses. While most of the medications exert their effects in the peripheral immune organs or blood stream, some also have the capacity to modulate the local immune responses and oligodendrocytes in the CNS.

In patients, Dimethyl fumarate (Tecfidera) decreased B cell CD40 expression (disrupted B-cell activation), decreased memory T cells, and decreased T cell proliferation and activation; resulting in lymphopenia (Linker and Gold, 2013). The mechanism of action of dimethyl fumarate within the CNS involves both Nrf-2-dependent as well as independent pathways for neuroprotection involving diminished neuroinflammation (Mills et al., 2018; Yadav et al., 2019). The Nrf2-dependent pathway promotes neuroprotection, oligodendrocyte survival, and decreases astrocyte activation (Linker et al., 2011; Kalinin et al., 2013; Wang et al., 2015; Zarrouk et al., 2017). The Nrf2-independent pathway also increases neuroprotection and decreases astrocyte activation, specifically reactive oxygen species production (i.e., Nitric Oxide) (Lin et al., 2011). Additionally, dimethyl fumarate targets innate immunity, in the form of microglia, resulting in diminished activation by the Nrf2 independent pathway (Parodi et al., 2015).

In patients, Fingolimod (Gilenya) suppresses migration of peripheral lymphocytes (Brinkmann et al., 2010; Francis et al., 2014). The mechanism of action of Fingolimod is the modulation of S1P receptor expression, most notably S1P1 receptor associated with lymphocytes, diminishing the number of T cells infiltrating into the CNS by retaining T cells in the lymph nodes (Brinkmann et al., 2002; Mandala et al., 2002; Fujino et al., 2003; Pham et al., 2008). Additionally, Fingolimod is neuroprotective, functioning within the CNS on neurons (Balatoni et al., 2007; Lee et al., 2010), oligodendrocyte lineage cells (Zhang et al., 2015), and decreasing the hyperactivity of reactive astrocytes (Choi et al., 2011). Of note, cumulatively Fingolimod has been shown to improve white matter integrity in relapsing remitting MS patients (Gurevich et al., 2018). Fingolimod can have "off target" effects as it can interact with multiple S1PR subtypes (S1PR1, S1PR3, S1PR4, and S1PR5) in a variety of tissues, including the heart (Chaudhry et al., 2017). The field has shifted to developing new therapies to mitigate these side effects, that selectively target subtype 1 of S1PR, yet this may diminish the neuroprotective capacity and immune suppression as S1PR5 is associated with oligodendrocyte function and natural killer cells (Chaudhry et al., 2017).

Natalizumab (Tysabri) is a monoclonal antibody against very late activating antigen (VLA)-α4 integrin and can bind to a majority of leukocytes, impeding cross over through the blood brain barrier into the CNS, thereby diminishing the aberrant heightened immune surveillance and inflammation (Stuve et al., 2006). However, use of Natalizumab in patients infected with John Cunningham virus (JCV) can result in

progressive multifocal leukoencephalopathy (PML) in a subset of patients (Clerico et al., 2017; Ho et al., 2017; Fragoso et al., 2019; Ryerson et al., 2019). JCV is an opportunistic virus causing oligodendrocyte destruction, demyelination, and eventually a detrimental inflammatory reaction. Natalizumab associated PML, leading to subsequent CNS inflammation and worsening of MS, underscores the interplay between the generation of free antigen (viral and myelin), T cell immune surveillance, and the rebalancing mechanisms of neuroinflammation. Overall, despite vast strides in disease modifying therapy (DMT) options for MS patients over the last few decades, substantial risk for adverse side effects remain with the existing therapies.

DISCUSSION/CONCLUSION

As Multiple Sclerosis is a syndrome with multiple clinical presentations and not a single disease entity, it is likely that both immune (outside-in) and neurodegenerative (inside-out) driven molecular pathways can initiate the etiopathogenesis of MS in different patients (**Table 1**). Additionally, it is important to consider that these two mechanisms are not mutually exclusive as, regardless of the initiating event, both Immune-mediated and neurodegenerative processes are important components of both types of models with the difference being the timing of the two processes. Each highlighted model has benefits yet limitations and not all pre-clinical models of MS were covered (Lassmann and Bradl, 2017). We highlighted both conventional as well as new experimental models for testing novel MS therapeutics, while exploring the underlying role of the adaptive and innate immune systems (**Table 2**). The three therapeutic targets for balancing immune dysfunction and preventing neurodegeneration necessary for effective amelioration of MS progression include: re-establishing self-tolerance, neuroprotection, and promotion of remyelination.

Chronic immunosuppression and immunomodulation are the most commonly used therapeutic strategies for MS, outside of symptom management. In addition to traditional disease-modifying therapies, immune reconstitution therapy (IRT) has emerged as a novel treatment paradigm (AlSharoqi et al., 2020; Derfuss et al., 2020). The latter is based on partial or full ablation of the immune system aiming to destroy self-reactive clones and restore normal function. Though attractive in principle, immune reconstitution at present is an uncontrolled process whose long-term efficacy and side effects remain to be established. Furthermore, IRT is a costly therapy that is available only in certain medical centers and typically reserved for patients with highly active disease. Immunotherapy based on re-establishing of self tolerance is likely to be more advantageous to patients in terms of disease control and avoidance of immunosuppressive side effects. Such strategy also may set the basis for personalized therapies of MS, where patient specific autoimmune responses are targeted by tolerizing agents. The Miller lab has recently demonstrated an effective means of ameliorating ongoing disease in EAE mouse models of MS by inducing tolerance in autoreactive $CD4^+$ T cells using intravenous (i.v.) infusion of 500 nM poly(lactic-*co*-glycolic acid) nanoparticles coupled with or encapsulating myelin peptides (Ag-PLG) that effectively reduces disease burden in relapsing-remitting (R-EAE) and in chronic-progressive (C-EAE) mouse models of experimental autoimmune encephalomyelitis (EAE) by reducing inflammatory cell activation and pro-inflammatory Th1/17 cytokine production (Getts et al., 2012, 2013; Hunter et al., 2014; McCarthy et al., 2017). Using myelin peptide-coupled autologous apoptotic leukocytes, we had previously demonstrated successful tolerance induction in MS patients (Lutterotti et al., 2013). Clinical testing of the Ag-PLG tolerance platform will be initiated in MS patients within the next year. We have recently shown, in a phase 1/2a trial in human celiac disease, safety and efficacy of PLG nanoparticles encapsulating gliadin (Kelly et al., 2019).

Interestingly, a recent single cell transcriptome study of oligodendrocyte lineage cells from the spinal cord of EAE mice indicated that oligodendrocytes and OPCs may not be passive targets of the immune attack, but rather involved in antigen-processing and presentation during the development of MS (Falcao et al., 2018). This possibility is supported by a previous study demonstrating that IFN-γ stimulated the production of chemokines from oligodendrocytes, while transgenic mice that suppresses oligodendrocyte responsiveness to IFN-γ developed an accelerated EAE onset (Balabanov et al., 2007).

The Popko lab has worked to enhance the protection of oligodendrocytes and myelin by augmenting the integrated stress response (ISR), a mechanism that protects endangered cells from inflammatory insults. Using a variety of mouse models of inflammatory demyelination, they have shown that genetic manipulations that compromise the ISR increase the susceptibility of oligodendrocytes in response to CNS inflammation (Lin et al., 2005, 2007) and that the genetic enhancement of the ISR, in contrast, provides increased protection to oligodendrocytes (Lin et al., 2008, 2013). Encouragingly, it has been shown that the ISR modulators, guanabenz and Sephin1, are able to protect oligodendrocytes against inflammatory stress through enhancing the ISR in MS mouse models (Way et al., 2015; Chen et al., 2019). Based on the "inside-out" theory, oligodendrocyte protection diminishes demyelination and reduces the generation of myelin debris, which likely decreases the exposure of myelin fragments and limits the autoimmune response. The success of these studies attests to the potential of oligodendrocyte protective therapeutics in MS.

At present, there are not any FDA approved therapies approved for myelin repair in MS despite successful pre-clinical trials. Utilizing both "outside-in" and "inside-out" models allows a comprehensive study of the multi-directional feedback between the CNS and periphery. As both immune dysregulation as well as inflammatory demyelination and neurodegeneration lead to disease progression, the field will need both "outside-in" and "inside-out" models to test single and combination therapies. Our collective goal as a field, of clinicians and scientists, is to improve patient outcomes and quality of life for those living with Multiple Sclerosis.

In summary, the availability and utilization of these diverse models allows the MS field a robust platform for developing novel therapeutics targeting the autoimmune response, neuronal stress and promoting myelin repair.

AUTHOR CONTRIBUTIONS

HET, YC, JP, RB, BP, and SDM conceived and outlined the manuscript and edited the manuscript. HET and YC wrote the manuscript. AR, HET, and SDM contributed to figures preparation. All authors contributed to the article and approved the submitted version.

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REFERENCES

Almuslehi, M. S. M., Sen, M. K., Shortland, P. J., Mahns, D. A., and Coorssen, J. R. (2020). CD8 T-cell recruitment into the central nervous system of cuprizone-fed mice: relevance to modeling the etiology of multiple sclerosis. *Front. Cell Neurosci.* 14:43. doi: 10.3389/fncel.2020.00043

AlSharoqi, I. A., Aljumah, M., Bohlega, S., Boz, C., Daif, A., El-Koussa, S., et al. (2020). Immune reconstitution therapy or continuous immunosuppression for the management of active relapsing-remitting multiple sclerosis patients? A Narrative Review. *Neurol. Ther.* 9, 55–66. doi: 10.1007/s40120-020-00187-3

Amor, S., Puentes, F., Baker, D., and Van Der Valk, P. (2010). Inflammation in neurodegenerative diseases. *Immunology* 129, 154–169.

Armstrong, R. C., Mierzwa, A. J., Marion, C. M., and Sullivan, G. M. (2016a). White matter involvement after TBI: clues to axon and myelin repair capacity. *Exp. Neurol.* 275(Pt 3), 328–333. doi: 10.1016/j.expneurol.2015.02.011

Armstrong, R. C., Mierzwa, A. J., Sullivan, G. M., and Sanchez, M. A. (2016b). Myelin and oligodendrocyte lineage cells in white matter pathology and plasticity after traumatic brain injury. *Neuropharmacology* 110, 654–659. doi: 10.1016/j.neuropharm.2015.04.029

Axthelm, M. K., Bourdette, D. N., Marracci, G. H., Su, W., Mullaney, E. T., Manoharan, M., et al. (2011). Japanese macaque encephalomyelitis: a spontaneous multiple sclerosis-like disease in a nonhuman primate. *Ann. Neurol.* 70, 362–373. doi: 10.1002/ana.22449

Bailey, S. L., Schreiner, B., Mcmahon, E. J., and Miller, S. D. (2007). CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4+ T(H)-17 cells in relapsing EAE. *Nat. Immunol.* 8, 172–180. doi: 10.1038/ni1430

Bakhti, M., Snaidero, N., Schneider, D., Aggarwal, S., Mobius, W., Janshoff, A., et al. (2013). Loss of electrostatic cell-surface repulsion mediates myelin membrane adhesion and compaction in the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3143–3148. doi: 10.1073/pnas.1220104110

Balabanov, R., Strand, K., Goswami, R., Mcmahon, E., Begolka, W., Miller, S. D., et al. (2007). Interferon-gamma-oligodendrocyte interactions in the regulation of experimental autoimmune encephalomyelitis. *J. Neurosci.* 27, 2013–2024. doi: 10.1523/jneurosci.4689-06.2007

Balatoni, B., Storch, M. K., Swoboda, E. M., Schonborn, V., Koziel, A., Lambrou, G. N., et al. (2007). FTY720 sustains and restores neuronal function in the DA rat model of MOG-induced experimental autoimmune encephalomyelitis. *Brain Res. Bull.* 74, 307–316. doi: 10.1016/j.brainresbull.2007.06.023

Barnett, M. H., and Prineas, J. W. (2004). Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol.* 55, 458–468. doi: 10.1002/ana.20016

Bazarian, J. J., Cernak, I., Noble-Haeusslein, L., Potolicchio, S., and Temkin, N. (2009). Long-term neurologic outcomes after traumatic brain injury. *J. Head Trauma Rehabil.* 24, 439–451. doi: 10.1097/htr.0b013e3181c15600

Beniac, D. R., Wood, D. D., Palaniyar, N., Ottensmeyer, F. P., Moscarello, M. A., and Harauz, G. (2000). Cryoelectron microscopy of protein-lipid complexes of human myelin basic protein charge isomers differing in degree of citrullination. *J. Struct. Biol.* 129, 80–95. doi: 10.1006/jsbi.1999.4200

Ben-Nun, A., Liblau, R. S., Cohen, L., Lehmann, D., Tournier-Lasserve, E., Rosenzweig, A., et al. (1991). Restricted T-cell receptor V beta gene usage by myelin basic protein-specific T-cell clones in multiple sclerosis: predominant genes vary in individuals. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2466–2470. doi: 10.1073/pnas.88.6.2466

Bhat, R., and Steinman, L. (2009). Innate and adaptive autoimmunity directed to the central nervous system. *Neuron* 64, 123–132. doi: 10.1016/j.neuron.2009.09.015

Blackwell, T. E., Butler, D. G., and Bell, J. A. (1983). Enterotoxemia in the goat: the humoral response and local tissue reaction following vaccination with two different bacterin-toxoids. *Can. J. Comp. Med.* 47, 127–132.

Blair, T. C., Manoharan, M., Rawlings-Rhea, S. D., Tagge, I., Kohama, S. G., Hollister-Smith, J., et al. (2016). Immunopathology of Japanese macaque encephalomyelitis is similar to multiple sclerosis. *J. Neuroimmunol.* 291, 1–10. doi: 10.1016/j.jneuroim.2015.11.026

Bossu, J. L., Wioland, L., Doussau, F., Isope, P., Popoff, M. R., and Poulain, B. (2020). Epsilon toxin from *Clostridium perfringens* causes inhibition of potassium inward rectifier (Kir) channels in oligodendrocytes. *Toxins* 12:36. doi: 10.3390/toxins12010036

Bradford, C. M., Ramos, I., Cross, A. K., Haddock, G., Mcquaid, S., Nicholas, A. P., et al. (2014). Localisation of citrullinated proteins in normal appearing white matter and lesions in the central nervous system in multiple sclerosis. *J. Neuroimmunol.* 273, 85–95. doi: 10.1016/j.jneuroim.2014.05.007

Brandao de Paiva, A. R., Pucci Filho, C. R., Porto, A. M., Feltrin, F. S., Kok, F., and Camargo, C. H. F. (2018). When multiple sclerosis and X-linked adrenoleukodystrophy are tangled: a challenging case. *Neurol. Clin. Pract.* 8, 156–158. doi: 10.1212/cpj.0000000000000431

Brinkmann, V., Billich, A., Baumruker, T., Heining, P., Schmouder, R., Francis, G., et al. (2010). Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat. Rev. Drug Discov.* 9, 883–897. doi: 10.1038/nrd3248

Brinkmann, V., Davis, M. D., Heise, C. E., Albert, R., Cottens, S., Hof, R., et al. (2002). The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J. Biol. Chem.* 277, 21453–21457. doi: 10.1074/jbc.c200176200

Burns, J., Littlefield, K., Gill, J., and Trotter, J. (1991). Autoantigen-induced self lysis of human myelin basic protein-specific T lymphocytes. *J. Neuroimmunol.* 35, 227–236. doi: 10.1016/0165-5728(91)90177-9

Cao, L., Goodin, R., Wood, D., Moscarello, M. A., and Whitaker, J. N. (1999). Rapid release and unusual stability of immunodominant peptide 45-89 from citrullinated myelin basic protein. *Biochemistry* 38, 6157–6163. doi: 10.1021/bi982960s

Caprariello, A. V., Rogers, J. A., Morgan, M. L., Hoghooghi, V., Plemel, J. R., Koebel, A., et al. (2018). Biochemically altered myelin triggers autoimmune demyelination. *Proc. Natl. Acad. Sci. U.S.A.* 115, 5528–5533. doi: 10.1073/pnas.1721115115

Chaudhry, B. Z., Cohen, J. A., and Conway, D. S. (2017). Sphingosine 1-Phosphate receptor modulators for the treatment of multiple sclerosis. *Neurotherapeutics* 14, 859–873. doi: 10.1007/s13311-017-0565-4

Chen, Y., Podojil, J. R., Kunjamma, R. B., Jones, J., Weiner, M., Lin, W., et al. (2019). Sephin1, which prolongs the integrated stress response, is a promising therapeutic for multiple sclerosis. *Brain* 142, 344–361. doi: 10.1093/brain/awy322

Choi, J. W., Gardell, S. E., Herr, D. R., Rivera, R., Lee, C. W., Noguchi, K., et al. (2011). FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. *Proc. Natl. Acad. Sci. U.S.A.* 108, 751–756. doi: 10.1073/pnas.1014154108

Chung, S., Fieremans, E., Wang, X., Kucukboyaci, N. E., Morton, C. J., Babb, J., et al. (2018). White matter tract integrity: an indicator of axonal pathology after mild traumatic brain injury. *J. Neurotrauma* 35, 1015–1020. doi: 10.1089/neu.2017.5320

Clerico, M., Artusi, C. A., Di Liberto, A., Rolla, S., Bardina, V., Barbero, P., et al. (2017). Long-term safety evaluation of natalizumab for the treatment of multiple sclerosis. *Expert Opin. Drug Saf.* 16, 963–972. doi: 10.1080/14740338.2017.1346082

Cloake, N. C., Yan, J., Aminian, A., Pender, M. P., and Greer, J. M. (2018). L1 mutations in patients with multiple sclerosis: identification of a new mutation and potential pathogenicity of the mutations. *J. Clin. Med.* 7:342. doi: 10.3390/jcm7100342

Collier, R. J. (2001). Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. *Toxicon* 39, 1793–1803. doi: 10.1016/s0041-0101(01)00165-9

Croxford, J. L., Olson, J. K., and Miller, S. D. (2002). Epitope spreading and molecular mimicry as triggers of autoimmunity in the Theiler's virus-induced demyelinating disease model of multiple sclerosis. *Autoimmun. Rev.* 1, 251–260. doi: 10.1016/s1568-9972(02)00080-0

Culpepper, W. J., Marrie, R. A., Langer-Gould, A., Wallin, M. T., Campbell, J. D., Nelson, L. M., et al. (2019). Validation of an algorithm for identifying MS cases in administrative health claims datasets. *Neurology* 92, e1016–e1028.

Daglas, M., Draxler, D. F., Ho, H., Mccutcheon, F., Galle, A., Au, A. E., et al. (2019). Activated CD8(+) T cells cause long-term neurological impairment after traumatic brain injury in mice. *Cell Rep.* 29, 1178.e6–1191.e6.

de Beer, M., Engelen, M., and Van Geel, B. M. (2014). Frequent occurrence of cerebral demyelination in adrenomyeloneuropathy. *Neurology* 83, 2227–2231. doi: 10.1212/wnl.0000000000001074

Dent, K. A., Christie, K. J., Bye, N., Basrai, H. S., Turbic, A., Habgood, M., et al. (2015). Oligodendrocyte birth and death following traumatic brain injury in adult mice. *PLoS One* 10:e0121541. doi: 10.1371/journal.pone.0121541

Derfuss, T., Mehling, M., Papadopoulou, A., Bar-Or, A., Cohen, J. A., and Kappos, L. (2020). Advances in oral immunomodulating therapies in relapsing multiple sclerosis. *Lancet Neurol.* 19, 336–347. doi: 10.1016/s1474-4422(19)30391-6

Doerflinger, N. H., Macklin, W. B., and Popko, B. (2003). Inducible site-specific recombination in myelinating cells. *Genesis* 35, 63–72. doi: 10.1002/gene.10154

Donovan, V., Kim, C., Anugerah, A. K., Coats, J. S., Oyoyo, U., Pardo, A. C., et al. (2014). Repeated mild traumatic brain injury results in long-term white-matter disruption. *J. Cereb. Blood Flow Metab.* 34, 715–723. doi: 10.1038/jcbfm.2014.6

Dorca-Arevalo, J., Soler-Jover, A., Gibert, M., Popoff, M. R., Martin-Satue, M., and Blasi, J. (2008). Binding of epsilon-toxin from *Clostridium perfringens* in the nervous system. *Vet. Microbiol.* 131, 14–25. doi: 10.1016/j.vetmic.2008.02.015

D'Souza, C. A., and Moscarello, M. A. (2006). Differences in susceptibility of MBP charge isomers to digestion by stromelysin-1 (MMP-3) and release of an immunodominant epitope. *Neurochem. Res.* 31, 1045–1054. doi: 10.1007/s11064-006-9116-9

Duan, Y., Sahley, C. L., and Muller, K. J. (2009). ATP and NO dually control migration of microglia to nerve lesions. *Dev. Neurobiol.* 69, 60–72. doi: 10.1002/dneu.20689

Esiri, M. M., and Reading, M. C. (1987). Macrophage populations associated with multiple sclerosis plaques. *Neuropathol. Appl. Neurobiol.* 13, 451–465. doi: 10.1111/j.1365-2990.1987.tb00074.x

Estep, R. D., Hansen, S. G., Rogers, K. S., Axthelm, M. K., and Wong, S. W. (2013). Genomic characterization of Japanese macaque rhadinovirus, a novel herpesvirus isolated from a nonhuman primate with a spontaneous inflammatory demyelinating disease. *J. Virol.* 87, 512–523. doi: 10.1128/jvi.02194-12

Falcao, A. M., Van Bruggen, D., Marques, S., Meijer, M., Jakel, S., Agirre, E., et al. (2018). Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. *Nat. Med.* 24, 1837–1844. doi: 10.1038/s41591-018-0236-y

Ferguson, B., Matyszak, M. K., Esiri, M. M., and Perry, V. H. (1997). Axonal damage in acute multiple sclerosis lesions. *Brain* 120(Pt 3), 393–399. doi: 10.1093/brain/120.3.393

Ferrer, I., Aubourg, P., and Pujol, A. (2010). General aspects and neuropathology of X-linked adrenoleukodystrophy. *Brain Pathol.* 20, 817–830. doi: 10.1111/j.1750-3639.2010.00390.x

Finnie, J. W. (1984). Ultrastructural changes in the brain of mice given *Clostridium perfringens* type D epsilon toxin. *J. Comp. Pathol.* 94, 445–452. doi: 10.1016/0021-9975(84)90031-8

Finnie, J. W. (2003). Pathogenesis of brain damage produced in sheep by *Clostridium perfringens* type D epsilon toxin: a review. *Aust. Vet. J.* 81, 219–221. doi: 10.1111/j.1751-0813.2003.tb11474.x

Fragoso, Y. D., Brooks, J. B. B., Eboni, A. C. B., Fezer, L., Da Gama, P. D., Gomes, S., et al. (2019). Seroconversion of JCV antibodies is strongly associated to natalizumab therapy. *J. Clin. Neurosci.* 61, 112–113. doi: 10.1016/j.jocn.2018.10.128

Francis, G., Kappos, L., O'connor, P., Collins, W., Tang, D., Mercier, F., et al. (2014). Temporal profile of lymphocyte counts and relationship with infections with fingolimod therapy. *Mult. Scler.* 20, 471–480. doi: 10.1177/1352458513500551

Franklin, R. J., and Ffrench-Constant, C. (2008). Remyelination in the CNS: from biology to therapy. *Nat. Rev. Neurosci.* 9, 839–855. doi: 10.1038/nrn2480

Fujino, M., Funeshima, N., Kitazawa, Y., Kimura, H., Amemiya, H., Suzuki, S., et al. (2003). Amelioration of experimental autoimmune encephalomyelitis in Lewis rats by FTY720 treatment. *J. Pharmacol. Exp. Ther.* 305, 70–77. doi: 10.1124/jpet.102.045658

Gerritse, K., Laman, J. D., Noelle, R. J., Aruffo, A., Ledbetter, J. A., Boersma, W. J., et al. (1996). CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2499–2504. doi: 10.1073/pnas.93.6.2499

Getts, D. R., Martin, A. J., Mccarthy, D. P., Terry, R. L., Hunter, Z. N., Yap, W. T., et al. (2012). Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis. *Nat. Biotechnol.* 30, 1217–1224. doi: 10.1038/nbt.2434

Getts, D. R., Mccarthy, D. P., and Miller, S. D. (2013). Exploiting apoptosis for therapeutic tolerance induction. *J. Immunol.* 191, 5341–5346. doi: 10.4049/jimmunol.1302070

Getts, D. R., Turley, D. M., Smith, C. E., Harp, C. T., Mccarthy, D., Feeney, E. M., et al. (2011). Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1+ and IL-10-producing splenic macrophages and maintained by T regulatory cells. *J. Immunol.* 187, 2405–2417. doi: 10.4049/jimmunol.1004175

Ghosh, A., Manrique-Hoyos, N., Voigt, A., Schulz, J. B., Kreutzfeldt, M., Merkler, D., et al. (2011). Targeted ablation of oligodendrocytes triggers axonal damage. *PLoS One* 6:e22735. doi: 10.1371/journal.pone.0022735

Giacci, M., and Fitzgerald, M. (2018). Oligodendroglia are particularly vulnerable to oxidative damage after neurotrauma in vivo. *J. Exp. Neurosci.* 12:1179069518810004.

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., and Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell* 140, 918–934. doi: 10.1016/j.cell.2010.02.016

Gritsch, S., Lu, J., Thilemann, S., Wortge, S., Mobius, W., Bruttger, J., et al. (2014). Oligodendrocyte ablation triggers central pain independently of innate or adaptive immune responses in mice. *Nat.Commun.* 5:5472.

Gurevich, M., Waknin, R., Stone, E., and Achiron, A. (2018). Fingolimod-improved axonal and myelin integrity of white matter tracts associated with multiple sclerosis-related functional impairments. *CNS Neurosci. Ther.* 24, 412–419. doi: 10.1111/cns.12796

Harp, C. T., Ireland, S., Davis, L. S., Remington, G., Cassidy, B., Cravens, P. D., et al. (2010). Memory B cells from a subset of treatment-naive relapsing-remitting multiple sclerosis patients elicit CD4(+) T-cell proliferation and IFN-gamma production in response to myelin basic protein and myelin oligodendrocyte glycoprotein. *Eur. J. Immunol.* 40, 2942–2956. doi: 10.1002/eji.201040516

Hausler, D., Hausser-Kinzel, S., Feldmann, L., Torke, S., Lepennetier, G., Bernard, C. C. A., et al. (2018). Functional characterization of reappearing B cells after anti-CD20 treatment of CNS autoimmune disease. *Proc. Natl. Acad. Sci. U.S.A.* 115, 9773–9778. doi: 10.1073/pnas.1810470115

Ho, J. K., Moser, H., Kishimoto, Y., and Hamilton, J. A. (1995). Interactions of a very long chain fatty acid with model membranes and serum albumin. Implications for the pathogenesis of adrenoleukodystrophy. *J. Clin. Invest.* 96, 1455–1463. doi: 10.1172/jci118182

Ho, P. R., Koendgen, H., Campbell, N., Haddock, B., Richman, S., and Chang, I. (2017). Risk of natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: a retrospective analysis of data from four clinical studies. *Lancet Neurol.* 16, 925–933. doi: 10.1016/s1474-4422(17)30282-x

Hudspeth, M. P., and Raymond, G. V. (2007). Immunopathogenesis of adrenoleukodystrophy: current understanding. *J. Neuroimmunol.* 182, 5–12. doi: 10.1016/j.jneuroim.2006.10.009

Hunter, Z., Mccarthy, D. P., Yap, W. T., Harp, C. T., Getts, D. R., Shea, L. D., et al. (2014). A biodegradable nanoparticle platform for the induction of antigen-specific immune tolerance for treatment of autoimmune disease. *ACS Nano* 8, 2148–2160. doi: 10.1021/nn405033r

International Multiple Sclerosis Genetics Consortium, A. H., Patsopoulos, N. A., Xifara, D. K., Davis, M. F., Kemppinen, A., et al. (2013). Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat. Genet.* 45, 1353–1360. doi: 10.1038/ng.2770

International Multiple Sclerosis Genetics Consortium (2019). Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science* 365:eaav7188. doi: 10.1126/science.aav7188

Ito, M., Blumberg, B. M., Mock, D. J., Goodman, A. D., Moser, A. B., Moser, H. W., et al. (2001). Potential environmental and host participants in the early white matter lesion of adreno-leukodystrophy: morphologic evidence for CD8 cytotoxic T cells, cytolysis of oligodendrocytes, and CD1-mediated lipid antigen presentation. *J. Neuropathol. Exp. Neurol.* 60, 1004–1019. doi: 10.1093/jnen/60.10.1004

Jakel, S., and Dimou, L. (2017). Glial cells and their function in the adult brain: a journey through the history of their ablation. *Front. Cell Neurosci.* 11:24. doi: 10.3389/fncel.2017.00024

Kalinin, S., Polak, P. E., Lin, S. X., Braun, D., Guizzetti, M., Zhang, X., et al. (2013). Dimethyl fumarate regulates histone deacetylase expression in astrocytes. *J. Neuroimmunol.* 263, 13–19. doi: 10.1016/j.jneuroim.2013.07.007

Kelly, C., Murray, J., Leffler, D., Bledsoe, A., Smithson, G., Podojil, J., et al. (2019). CNP-101 prevents gluten challenge induced immune activation in adults with celiac disease. *Proc. United Eur. Gastroenterol.* 7. Available online at: https://ueg.eu/library/cnp-101-prevents-gluten-challenge-induced-immune-activation-in-adults-with-celiac-disease/208600

Kemp, S., Huffnagel, I. C., Linthorst, G. E., Wanders, R. J., and Engelen, M. (2016). Adrenoleukodystrophy - neuroendocrine pathogenesis and redefinition of natural history. *Nat. Rev. Endocrinol.* 12, 606–615. doi: 10.1038/nrendo.2016.90

Korenke, G. C., Pouwels, P. J., Frahm, J., Hunneman, D. H., Stoeckler, S., Krasemann, E., et al. (1996). Arrested cerebral adrenoleukodystrophy: a clinical and proton magnetic resonance spectroscopy study in three patients. *Pediatr. Neurol.* 15, 103–107. doi: 10.1016/0887-8994(95)00156-5

Kostic, M., Zivkovic, N., and Stojanovic, I. (2013). Multiple sclerosis and glutamate excitotoxicity. *Rev. Neurosci.* 24, 71–88.

Kotelnikova, E., Kiani, N. A., Messinis, D., Pertsovskaya, I., Pliaka, V., Bernardo-Faura, M., et al. (2019). MAPK pathway and B cells overactivation in multiple sclerosis revealed by phosphoproteomics and genomic analysis. *Proc. Natl. Acad. Sci. U.S.A.* 116, 9671–9676. doi: 10.1073/pnas.1818347116

Lassmann, H., and Bradl, M. (2017). Multiple sclerosis: experimental models and reality. *Acta Neuropathol.* 133, 223–244. doi: 10.1007/s00401-016-1631-4

Lee, C. W., Choi, J. W., and Chun, J. (2010). Neurological S1P signaling as an emerging mechanism of action of oral FTY720 (fingolimod) in multiple sclerosis. *Arch. Pharm. Res.* 33, 1567–1574. doi: 10.1007/s12272-010-1008-5

Lehmann, P. V., Forsthuber, T., Miller, A., and Sercarz, E. E. (1992). Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature* 358, 155–157. doi: 10.1038/358155a0

Lehmann, P. V., Sercarz, E. E., Forsthuber, T., Dayan, C. M., and Gammon, G. (1993). Determinant spreading and the dynamics of the autoimmune T-cell repertoire. *Immunol. Today* 14, 203–208. doi: 10.1016/0167-5699(93)90163-f

Lehmann, P. V., Targoni, O. S., and Forsthuber, T. G. (1998). Shifting T-cell activation thresholds in autoimmunity and determinant spreading. *Immunol. Rev.* 164, 53–61. doi: 10.1111/j.1600-065x.1998.tb01207.x

Lin, S. X., Lisi, L., Dello Russo, C., Polak, P. E., Sharp, A., Weinberg, G., et al. (2011). The anti-inflammatory effects of dimethyl fumarate in astrocytes involve glutathione and haem oxygenase-1. *ASN Neuro* 3:e00055.

Lin, W., Bailey, S. L., Ho, H., Harding, H. P., Ron, D., Miller, S. D., et al. (2007). The integrated stress response prevents demyelination by protecting oligodendrocytes against immune-mediated damage. *J. Clin. Invest.* 117, 448–456. doi: 10.1172/jci29571

Lin, W., Harding, H. P., Ron, D., and Popko, B. (2005). Endoplasmic reticulum stress modulates the response of myelinating oligodendrocytes to the immune cytokine interferon-gamma. *J. Cell Biol.* 169, 603–612. doi: 10.1083/jcb.200502086

Lin, W., Kunkler, P. E., Harding, H. P., Ron, D., Kraig, R. P., and Popko, B. (2008). Enhanced integrated stress response promotes myelinating oligodendrocyte survival in response to interferon-gamma. *Am. J. Pathol.* 173, 1508–1517. doi: 10.2353/ajpath.2008.080449

Lin, W., Lin, Y., Li, J., Fenstermaker, A. G., Way, S. W., Clayton, B., et al. (2013). Oligodendrocyte-specific activation of PERK signaling protects mice against experimental autoimmune encephalomyelitis. *J. Neurosci.* 33, 5980–5991. doi: 10.1523/jneurosci.1636-12.2013

Linden, J. R., Ma, Y., Zhao, B., Harris, J. M., Rumah, K. R., Schaeren-Wiemers, N., et al. (2015). *Clostridium perfringens* epsilon toxin causes selective death of mature oligodendrocytes and central nervous system demyelination. *mBio* 6:e02513.

Linker, R. A., and Gold, R. (2013). Dimethyl fumarate for treatment of multiple sclerosis: mechanism of action, effectiveness, and side effects. *Curr. Neurol. Neurosci. Rep.* 13:394.

Linker, R. A., Lee, D. H., Ryan, S., Van Dam, A. M., Conrad, R., Bista, P., et al. (2011). Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* 134, 678–692. doi: 10.1093/brain/awq386

Locatelli, G., Wortge, S., Buch, T., Ingold, B., Frommer, F., Sobottka, B., et al. (2012). Primary oligodendrocyte death does not elicit anti-CNS immunity. *Nat. Neurosci.* 15, 543–550. doi: 10.1038/nn.3062

Lonchamp, E., Dupont, J. L., Wioland, L., Courjaret, R., Mbebi-Liegeois, C., Jover, E., et al. (2010). *Clostridium perfringens* epsilon toxin targets granule cells in the mouse cerebellum and stimulates glutamate release. *PLoS One* 5:e13046. doi: 10.1371/journal.pone.0013046

Lotocki, G., De Rivero Vaccari, J. P., Alonso, O., Molano, J. S., Nixon, R., Safavi, P., et al. (2011). Oligodendrocyte vulnerability following traumatic brain injury in rats. *Neurosci. Lett.* 499, 143–148. doi: 10.1016/j.neulet.2011.05.056

Luo, X., Miller, S. D., and Shea, L. D. (2016). Immune tolerance for autoimmune disease and cell transplantation. *Annu. Rev. Biomed. Eng.* 18, 181–205. doi: 10.1146/annurev-bioeng-110315-020137

Lutterotti, A., Yousef, S., Sputtek, A., Sturner, K. H., Stellmann, J. P., Breiden, P., et al. (2013). Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis. *Sci. Transl. Med.* 5:188ra175.

Mack, C. L., Vanderlugt-Castaneda, C. L., Neville, K. L., and Miller, S. D. (2003). Microglia are activated to become competent antigen presenting and effector cells in the inflammatory environment of the Theiler's virus model of multiple sclerosis. *J. Neuroimmunol.* 144, 68–79. doi: 10.1016/j.jneuroim.2003.08.032

Manconi, B., Liori, B., Cabras, T., Vincenzoni, F., Iavarone, F., Lorefice, L., et al. (2018). Top-down proteomic profiling of human saliva in multiple sclerosis patients. *J. Proteomics* 187, 212–222. doi: 10.1016/j.jprot.2018.07.019

Mandala, S., Hajdu, R., Bergstrom, J., Quackenbush, E., Xie, J., Milligan, J., et al. (2002). Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* 296, 346–349. doi: 10.1126/science.1070238

Matsushima, G. K., and Morell, P. (2001). The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol.* 11, 107–116. doi: 10.1111/j.1750-3639.2001.tb00385.x

McCarthy, D. P., Hunter, Z. N., Chackerian, B., Shea, L. D., and Miller, S. D. (2014). Targeted immunomodulation using antigen-conjugated nanoparticles. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 6, 298–315. doi: 10.1002/wnan.1263

McCarthy, D. P., Yap, J. W., Harp, C. T., Song, W. K., Chen, J., Pearson, R. M., et al. (2017). An antigen-encapsulating nanoparticle platform for TH1/17 immune tolerance therapy. *Nanomedicine* 13, 191–200. doi: 10.1016/j.nano.2016.09.007

McFarland, H. F., and Martin, R. (2007). Multiple sclerosis: a complicated picture of autoimmunity. *Nat. Immunol.* 8, 913–919. doi: 10.1038/ni1507

McMahon, E. J., Bailey, S. L., Castenada, C. V., Waldner, H., and Miller, S. D. (2005). Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat. Med.* 11, 335–339. doi: 10.1038/nm1202

McRae, B. L., Vanderlugt, C. L., Dal Canto, M. C., and Miller, S. D. (1995). Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 182, 75–85. doi: 10.1084/jem.182.1.75

Mierzwa, A. J., Marion, C. M., Sullivan, G. M., Mcdaniel, D. P., and Armstrong, R. C. (2015). Components of myelin damage and repair in the progression of white matter pathology after mild traumatic brain injury. *J. Neuropathol. Exp. Neurol.* 74, 218–232. doi: 10.1097/nen.0000000000000165

Miller, D. H., and Leary, S. M. (2007). Primary-progressive multiple sclerosis. *Lancet Neurol.* 6, 903–912.

Miller, S. D., Mcrae, B. L., Vanderlugt, C. L., Nikcevich, K. M., Pope, J. G., Pope, L., et al. (1995). Evolution of the T-cell repertoire during the course of experimental immune-mediated demyelinating diseases. *Immunol. Rev.* 144, 225–244. doi: 10.1111/j.1600-065x.1995.tb00071.x

Miller, S. D., Vanderlugt, C. L., Begolka, W. S., Pao, W., Yauch, R. L., Neville, K. L., et al. (1997). Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat. Med.* 3, 1133–1136. doi: 10.1038/nm1097-1133

Mills, E. A., Ogrodnik, M. A., Plave, A., and Mao-Draayer, Y. (2018). Emerging understanding of the mechanism of action for dimethyl fumarate in the treatment of multiple sclerosis. *Front. Neurol.* 9:5. doi: 10.3389/fneur.2018.00005

Moon, J. J., Chu, H. H., Pepper, M., Mcsorley, S. J., Jameson, S. C., Kedl, R. M., et al. (2007). Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 27, 203–213. doi: 10.1016/j.immuni.2007.07.007

Moscarello, M. A., Mastronardi, F. G., and Wood, D. D. (2007). The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple sclerosis. *Neurochem. Res.* 32, 251–256. doi: 10.1007/s11064-006-9144-5

Moscarello, M. A., Wood, D. D., Ackerley, C., and Boulias, C. (1994). Myelin in multiple sclerosis is developmentally immature. *J. Clin. Invest.* 94, 146–154. doi: 10.1172/jci117300

Moser, H. W., Mahmood, A., and Raymond, G. V. (2007). X-linked adrenoleukodystrophy. *Nat. Clin. Pract. Neurol.* 3, 140–151.

Moser, H. W., Moser, A. B., Smith, K. D., Bergin, A., Borel, J., Shankroff, J., et al. (1992). Adrenoleukodystrophy: phenotypic variability and implications for therapy. *J. Inherit. Metab. Dis.* 15, 645–664. doi: 10.1007/bf01799621

Munz, C., Lunemann, J. D., Getts, M. T., and Miller, S. D. (2009). Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat. Rev. Immunol.* 9, 246–258. doi: 10.1038/nri2527

Murrell, T. G., O'donoghue, P. J., and Ellis, T. (1986). A review of the sheep-multiple sclerosis connection. *Med. Hypotheses* 19, 27–39. doi: 10.1016/0306-9877(86)90134-9

Musse, A. A., Boggs, J. M., and Harauz, G. (2006). Deimination of membrane-bound myelin basic protein in multiple sclerosis exposes an immunodominant epitope. *Proc. Natl. Acad. Sci. U.S.A.* 103, 4422–4427. doi: 10.1073/pnas.0509158103

Nave, K. A. (2010). Myelination and support of axonal integrity by glia. *Nature* 468, 244–252. doi: 10.1038/nature09614

Nelson, L. M., Wallin, M. T., Marrie, R. A., Culpepper, W. J., Langer-Gould, A., Campbell, J., et al. (2019). A new way to estimate neurologic disease prevalence in the United States: Illustrated with MS. *Neurology* 92, 469–480. doi: 10.1212/wnl.0000000000007044

Olson, J. K., Croxford, J. L., Calenoff, M. A., Dal Canto, M. C., and Miller, S. D. (2001). A virus-induced molecular mimicry model of multiple sclerosis. *J. Clin. Invest.* 108, 311–318. doi: 10.1172/jci200113032

Oluich, L. J., Stratton, J. A., Xing, Y. L., Ng, S. W., Cate, H. S., Sah, P., et al. (2012). Targeted ablation of oligodendrocytes induces axonal pathology independent of overt demyelination. *J. Neurosci.* 32, 8317–8330. doi: 10.1523/jneurosci.1053-12.2012

O'Phelan, K. H., Otoshi, C. K., Ernst, T., and Chang, L. (2018). Common patterns of regional brain injury detectable by diffusion tensor imaging in otherwise normal-appearing white matter in patients with early moderate to severe traumatic brain injury. *J. Neurotrauma* 35, 739–749. doi: 10.1089/neu.2016.4944

Parodi, B., Rossi, S., Morando, S., Cordano, C., Bragoni, A., Motta, C., et al. (2015). Fumarates modulate microglia activation through a novel HCAR2 signaling pathway and rescue synaptic dysregulation in inflamed CNS. *Acta Neuropathol.* 130, 279–295. doi: 10.1007/s00401-015-1422-3

Partridge, M. A., Gopinath, S., Myers, S. J., and Coorssen, J. R. (2016). An initial top-down proteomic analysis of the standard cuprizone mouse model of multiple sclerosis. *J. Chem. Biol.* 9, 9–18. doi: 10.1007/s12154-015-0138-0

Pearson, R. M., Casey, L. M., Hughes, K. R., Miller, S. D., and Shea, L. D. (2017). In vivo reprogramming of immune cells: technologies for induction of antigen-specific tolerance. *Adv. Drug Deliv. Rev.* 114, 240–255. doi: 10.1016/j.addr.2017.04.005

Pearson, R. M., Podojil, J. R., Shea, L. D., King, N. J. C., Miller, S. D., and Getts, D. R. (2019). Overcoming challenges in treating autoimmuntity: development of tolerogenic immune-modifying nanoparticles. *Nanomedicine* 18, 282–291. doi: 10.1016/j.nano.2018.10.001

Pham, T. H., Okada, T., Matloubian, M., Lo, C. G., and Cyster, J. G. (2008). S1P1 receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity* 28, 122–133. doi: 10.1016/j.immuni.2007.11.017

Podojil, J. R., and Miller, S. D. (2009). Molecular mechanisms of T-cell receptor and costimulatory molecule ligation/blockade in autoimmune disease therapy. *Immunol. Rev.* 229, 337–355. doi: 10.1111/j.1600-065x.2009.00773.x

Popoff, M. R. (2011). Epsilon toxin: a fascinating pore-forming toxin. *FEBS J.* 278, 4602–4615. doi: 10.1111/j.1742-4658.2011.08145.x

Powers, J. M., Liu, Y., Moser, A. B., and Moser, H. W. (1992). The inflammatory myelinopathy of adreno-leukodystrophy: cells, effector molecules, and pathogenetic implications. *J. Neuropathol. Exp. Neurol.* 51, 630–643. doi: 10.1097/00005072-199211000-00007

Prasad, S., Xu, D., and Miller, S. D. (2012). Tolerance strategies employing antigen-coupled apoptotic cells and carboxylated PLG nanoparticles for the treatment of type 1 diabetes. *Rev. Diabet Stud.* 9, 319–327. doi: 10.1900/rds.2012.9.319

Prineas, J. W., Kwon, E. E., Cho, E. S., Sharer, L. R., Barnett, M. H., Oleszak, E. L., et al. (2001). Immunopathology of secondary-progressive multiple sclerosis. *Ann. Neurol.* 50, 646–657.

Prineas, J. W., and Parratt, J. D. (2012). Oligodendrocytes and the early multiple sclerosis lesion. *Ann. Neurol.* 72, 18–31. doi: 10.1002/ana.23634

Prinz, M., and Kalinke, U. (2010). New lessons about old molecules: how type I interferons shape Th1/Th17-mediated autoimmunity in the CNS. *Trends Mol. Med.* 16, 379–386. doi: 10.1016/j.molmed.2010.06.001

Raijmakers, R., Vogelzangs, J., Croxford, J. L., Wesseling, P., Van Venrooij, W. J., and Pruijn, G. J. (2005). Citrullination of central nervous system proteins during the development of experimental autoimmune encephalomyelitis. *J. Comp. Neurol.* 486, 243–253. doi: 10.1002/cne.20529

Raymond, G. V., Seidman, R., Monteith, T. S., Kolodny, E., Sathe, S., Mahmood, A., et al. (2010). Head trauma can initiate the onset of adreno-leukodystrophy. *J. Neurol. Sci.* 290, 70–74. doi: 10.1016/j.jns.2009.11.005

Robinson, A. P., Harp, C. T., Noronha, A., and Miller, S. D. (2014). The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. *Handb. Clin. Neurol.* 122, 173–189. doi: 10.1016/b978-0-444-52001-2.00008-x

Rodgers, J. M., Robinson, A. P., and Miller, S. D. (2013). Strategies for protecting oligodendrocytes and enhancing remyelination in multiple sclerosis. *Discov. Med.* 16, 53–63.

Rumah, K. R., Linden, J., Fischetti, V. A., and Vartanian, T. (2013). Isolation of *Clostridium perfringens* type B in an individual at first clinical presentation of multiple sclerosis provides clues for environmental triggers of the disease. *PLoS One* 8:e76359. doi: 10.1371/journal.pone.0076359

Rumah, K. R., Ma, Y., Linden, J. R., Oo, M. L., Anrather, J., Schaeren-Wiemers, N., et al. (2015). The myelin and lymphocyte protein MAL is required for binding and activity of *Clostridium perfringens* epsilon-toxin. *PLoS Pathog.* 11:e1004896. doi: 10.1371/journal.ppat.1004896

Rutgers, D. R., Fillard, P., Paradot, G., Tadie, M., Lasjaunias, P., and Ducreux, D. (2008). Diffusion tensor imaging characteristics of the corpus callosum in mild, moderate, and severe traumatic brain injury. *AJNR Am. J. Neuroradiol.* 29, 1730–1735. doi: 10.3174/ajnr.a1213

Ryerson, L. Z., Foley, J., Chang, I., Kister, I., Cutter, G., Metzger, R. R., et al. (2019). Risk of natalizumab-associated PML in patients with MS is reduced with extended interval dosing. *Neurology* 93, e1452–e1462.

Schwartz, M., and Raposo, C. (2014). Protective autoimmunity: a unifying model for the immune network involved in CNS repair. *Neuroscientist* 20, 343–358. doi: 10.1177/1073858413516799

Sen, M. K., Almuslehi, M. S. M., Gyengesi, E., Myers, S. J., Shortland, P. J., Mahns, D. A., et al. (2019a). Suppression of the peripheral immune system limits the central immune response following cuprizone-feeding: relevance to modelling multiple sclerosis. *Cells* 8:1314. doi: 10.3390/cells8111314

Sen, M. K., Mahns, D. A., Coorssen, J. R., and Shortland, P. J. (2019b). Behavioural phenotypes in the cuprizone model of central nervous system demyelination. *Neurosci. Biobehav. Rev.* 107, 23–46. doi: 10.1016/j.neubiorev.2019.08.008

Sharma, S., Ifergan, I., Kurz, J. E., Linsenmeier, R. A., Xu, D., Cooper, J. G., et al. (2020). Intravenous immunomodulatory nanoparticle treatment for traumatic brain injury. *Ann. Neurol.* 87, 442–455. doi: 10.1002/ana.25675

Sidaway, P. (2017). Multiple sclerosis: concussion during adolescence linked to increased risk of MS. *Nat. Rev. Neurol.* 13:640. doi: 10.1038/nrneurol.2017.135

Singh, J., Khan, M., and Singh, I. (2009). Silencing of Abcd1 and Abcd2 genes sensitizes astrocytes for inflammation: implication for X-adrenoleukodystrophy. *J. Lipid Res.* 50, 135–147. doi: 10.1194/jlr.m800321-jlr200

Soderstrom, M., Link, H., Sun, J. B., Fredrikson, S., Kostulas, V., Hojeberg, B., et al. (1993). T cells recognizing multiple peptides of myelin basic protein are found in blood and enriched in cerebrospinal fluid in optic neuritis and multiple sclerosis. *Scand. J. Immunol.* 37, 355–368. doi: 10.1111/j.1365-3083.1993.tb02565.x

Solti, I., Kvell, K., Talaber, G., Veto, S., Acs, P., Gallyas, F., et al. (2015). Thymic atrophy and apoptosis of CD4+CD8+ thymocytes in the cuprizone model of multiple sclerosis. *PLoS One* 10:e0129217. doi: 10.1371/journal.pone.0129217

Sospedra, M., and Martin, R. (2005). Immunology of multiple sclerosis. *Annu. Rev. Immunol.* 23, 683–747.

Stadelmann, C. (2011). Multiple sclerosis as a neurodegenerative disease: pathology, mechanisms and therapeutic implications. *Curr. Opin. Neurol.* 24, 224–229. doi: 10.1097/wco.0b013e328346056f

Steinman, L. (2009). A molecular trio in relapse and remission in multiple sclerosis. *Nat. Rev. Immunol.* 9, 440–447. doi: 10.1038/nri2548

Steinman, L., Martin, R., Bernard, C., Conlon, P., and Oksenberg, J. R. (2002). Multiple sclerosis: deeper understanding of its pathogenesis reveals new targets for therapy. *Annu. Rev. Neurosci.* 25, 491–505. doi: 10.1146/annurev.neuro.25.112701.142913

Stuve, O., Marra, C. M., Jerome, K. R., Cook, L., Cravens, P. D., Cepok, S., et al. (2006). Immune surveillance in multiple sclerosis patients treated with natalizumab. *Ann. Neurol.* 59, 743–747.

Stys, P. K., Zamponi, G. W., Van Minnen, J., and Geurts, J. J. (2012). Will the real multiple sclerosis please stand up? *Nat. Rev. Neurosci.* 13, 507–514. doi: 10.1038/nrn3275

Sun, J., Link, H., Olsson, T., Xiao, B. G., Andersson, G., Ekre, H. P., et al. (1991). T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. *J. Immunol.* 146, 1490–1495.

Tamai, E., Ishida, T., Miyata, S., Matsushita, O., Suda, H., Kobayashi, S., et al. (2003). Accumulation of *Clostridium perfringens* epsilon-toxin in the mouse kidney and its possible biological significance. *Infect. Immun.* 71, 5371–5375. doi: 10.1128/iai.71.9.5371-5375.2003

Terry, R. L., Ifergan, I., and Miller, S. D. (2016). Experimental autoimmune encephalomyelitis in mice. *Methods Mol. Biol.* 1304, 145–160.

Theiler, M., and Gard, S. (1940). Encephalomyelitis of Mice : iii. Epidemiology. *J. Exp. Med.* 72, 79–90.

Traka, M., Arasi, K., Avila, R. L., Podojil, J. R., Christakos, A., Miller, S. D., et al. (2010). A genetic mouse model of adult-onset, pervasive central nervous system demyelination with robust remyelination. *Brain* 133, 3017–3029. doi: 10.1093/brain/awq247

Traka, M., Podojil, J. R., Mccarthy, D. P., Miller, S. D., and Popko, B. (2016). Oligodendrocyte death results in immune-mediated CNS demyelination. *Nat. Neurosci.* 19, 65–74. doi: 10.1038/nn.4193

Trapp, B. D., and Nave, K. A. (2008). Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 31, 247–269. doi: 10.1146/annurev.neuro.30.051606.094313

Trotter, J. L., Hickey, W. F., Van Der Veen, R. C., and Sulze, L. (1991). Peripheral blood mononuclear cells from multiple sclerosis patients recognize myelin proteolipid protein and selected peptides. *J. Neuroimmunol.* 33, 55–62. doi: 10.1016/0165-5728(91)90034-5

Uzal, F. A., Kelly, W. R., Morris, W. E., Bermudez, J., and Baison, M. (2004). The pathology of peracute experimental *Clostridium perfringens* type D enterotoxemia in sheep. *J. Vet. Diagn. Invest.* 16, 403–411. doi: 10.1177/104063870401600506

Uzal, F. A., and Songer, J. G. (2008). Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. *J. Vet. Diagn. Invest.* 20, 253–265.

Vanderlugt, C. L., and Miller, S. D. (2002). Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat. Rev. Immunol.* 2, 85–95. doi: 10.1038/nri724

Veillette, A., Zuniga-Pflucker, J. C., Bolen, J. B., and Kruisbeek, A. M. (1989). Engagement of CD4 and CD8 expressed on immature thymocytes induces activation of intracellular tyrosine phosphorylation pathways. *J. Exp. Med.* 170, 1671–1680. doi: 10.1084/jem.170.5.1671

Vossenaar, E. R., Zendman, A. J., Van Venrooij, W. J., and Pruijn, G. J. (2003). PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioessays* 25, 1106–1118. doi: 10.1002/bies.10357

Wagley, S., Bokori-Brown, M., Morcrette, H., Malaspina, A., D'arcy, C., Gnanapavan, S., et al. (2019). Evidence of *Clostridium perfringens* epsilon toxin associated with multiple sclerosis. *Mult. Scler* 25, 653–660. doi: 10.1177/1352458518767327

Wallin, M. T., Culpepper, W. J., Campbell, J. D., Nelson, L. M., Langer-Gould, A., Marrie, R. A., et al. (2019). The prevalence of MS in the United States: A population-based estimate using health claims data. *Neurology* 92, e1029–e1040.

Wang, Q., Chuikov, S., Taitano, S., Wu, Q., Rastogi, A., Tuck, S. J., et al. (2015). Dimethyl fumarate protects neural stem/progenitor cells and neurons from oxidative damage through Nrf2-ERK1/2 MAPK Pathway. *Int. J. Mol. Sci.* 16, 13885–13907. doi: 10.3390/ijms160613885

Warshawsky, I., Rudick, R. A., Staugaitis, S. M., and Natowicz, M. R. (2005). Primary progressive multiple sclerosis as a phenotype of a PLP1 gene mutation. *Ann. Neurol.* 58, 470–473. doi: 10.1002/ana.20601

Way, S. W., Podojil, J. R., Clayton, B. L., Zaremba, A., Collins, T. L., Kunjamma, R. B., et al. (2015). Pharmaceutical integrated stress response enhancement protects oligodendrocytes and provides a potential multiple sclerosis therapeutic. *Nat. Commun.* 6:6532.

Weiner, H. L. (2009). The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? *Ann. Neurol.* 65, 239–248. doi: 10.1002/ana.21640

Weller, M., Liedtke, W., Petersen, D., Opitz, H., and Poremba, M. (1992). Very-late-onset adrenoleukodystrophy: possible precipitation of demyelination by cerebral contusion. *Neurology* 42, 367–370. doi: 10.1212/wnl.42.2.367

Wetzig, R., Hanson, D. G., Miller, S. D., and Claman, H. N. (1979). Binding of ovalbumin to mouse spleen cells with and without carbodiimide. *J. Immunol. Methods* 28, 361–368. doi: 10.1016/0022-1759(79)90201-1

Windhagen, A., Newcombe, J., Dangond, F., Strand, C., Woodroofe, M. N., Cuzner, M. L., et al. (1995). Expression of costimulatory molecules B7-1 (CD80). B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. *J. Exp. Med.* 182, 1985–1996. doi: 10.1084/jem.182.6.1985

Wioland, L., Dupont, J. L., Doussau, F., Gaillard, S., Heid, F., Isope, P., et al. (2015). Epsilon toxin from *Clostridium perfringens* acts on oligodendrocytes without forming pores, and causes demyelination. *Cell Microbiol.* 17, 369–388. doi: 10.1111/cmi.12373

Wood, D. D., Bilbao, J. M., O'connors, P., and Moscarello, M. A. (1996). Acute multiple sclerosis (Marburg type) is associated with developmentally immature myelin basic protein. *Ann. Neurol.* 40, 18–24. doi: 10.1002/ana.410400106

Yadav, S. K., Soin, D., Ito, K., and Dhib-Jalbut, S. (2019). Insight into the mechanism of action of dimethyl fumarate in multiple sclerosis. *J. Mol. Med.* 97, 463–472. doi: 10.1007/s00109-019-01761-5

Zarrouk, A., Nury, T., Karym, E. M., Vejux, A., Sghaier, R., Gondcaille, C., et al. (2017). Attenuation of 7-ketocholesterol-induced overproduction of reactive oxygen species, apoptosis, and autophagy by dimethyl fumarate on 158N murine oligodendrocytes. *J. Steroid. Biochem. Mol. Biol.* 169, 29–38. doi: 10.1016/j.jsbmb.2016.02.024

Zhang, J., Zhang, Z. G., Li, Y., Ding, X., Shang, X., Lu, M., et al. (2015). Fingolimod treatment promotes proliferation and differentiation of oligodendrocyte progenitor cells in mice with experimental autoimmune encephalomyelitis. *Neurobiol. Dis.* 76, 57–66. doi: 10.1016/j.nbd.2015.01.006

Zhang, Y., Burger, D., Saruhan, G., Jeannet, M., and Steck, A. J. (1993). The T-lymphocyte response against myelin-associated glycoprotein and myelin basic protein in patients with multiple sclerosis. *Neurology* 43, 403–407. doi: 10.1212/wnl.43.2.403

Zrzavy, T., Hametner, S., Wimmer, I., Butovsky, O., Weiner, H. L., and Lassmann, H. (2017). Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* 140, 1900–1913. doi: 10.1093/brain/awx113

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REVIEW
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Autophagy in Multiple Sclerosis: Two Sides of the Same Coin

*Chairi Misrielal[1], Mario Mauthe[2], Fulvio Reggiori[2] and Bart J. L. Eggen[1]**

[1] Molecular Neurobiology, Department of Biomedical Sciences of Cells and Systems, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, [2] Molecular Cell Biology, Department of Biomedical Sciences of Cells and Systems, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Multiple sclerosis (MS) is a complex auto-immune disorder of the central nervous system (CNS) that involves a range of CNS and immune cells. MS is characterized by chronic neuroinflammation, demyelination, and neuronal loss, but the molecular causes of this disease remain poorly understood. One cellular process that could provide insight into MS pathophysiology and also be a possible therapeutic avenue, is autophagy. Autophagy is an intracellular degradative pathway essential to maintain cellular homeostasis, particularly in neurons as defects in autophagy lead to neurodegeneration. One of the functions of autophagy is to maintain cellular homeostasis by eliminating defective or superfluous proteins, complexes, and organelles, preventing the accumulation of potentially cytotoxic damage. Importantly, there is also an intimate and intricate interplay between autophagy and multiple aspects of both innate and adaptive immunity. Thus, autophagy is implicated in two of the main hallmarks of MS, neurodegeneration, and inflammation, making it especially important to understand how this pathway contributes to MS manifestation and progression. This review summarizes the current knowledge about autophagy in MS, in particular how it contributes to our understanding of MS pathology and its potential as a novel therapeutic target.

Keywords: autophagy, multiple sclerosis, neurodegeneration, inflammation, resolution

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***Correspondence:**
Bart J. L. Eggen
b.j.l.eggen@umcg.nl

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INTRODUCTION

Autophagy is a lysosomal degradation system for damaged or unwanted organelles, aggregates, and long-lived proteins, which is important for cellular homeostasis (Choi et al., 2013). This process is responsible for nutrient supply under starved conditions by recycling the metabolites composing cellular components (Lahiri et al., 2019). Autophagy is also involved in a multitude of other physiological functions, including the regulation of innate and adaptive immune responses (Beau et al., 2011; Levine et al., 2011). In recent years, the involvement of autophagy in several pathological conditions, such as neurodegenerative disorders and autoimmune diseases, has become evident as well (Mizushima et al., 2008; Law et al., 2010; Ravikumar et al., 2010; van Beek et al., 2018; Yin et al., 2018; Levine and Kroemer, 2019).

Multiple sclerosis (MS) is a demyelinating auto-immune disorder of the central nervous system (CNS), which is driven by a complex interaction between environmental, genetic, and immunological factors. MS is characterized by the interplay of neuroinflammatory and

neurodegenerative processes, resulting in progressive disability of patients (Dyment et al., 2006; Sawcer et al., 2011; Dobson and Giovannoni, 2019). Although this disease has been viewed for a long time as a T-cell-mediated autoimmune disease, recent investigations have uncovered that MS is a complex disorder that involves many cell types, including both other immune cells, such as dendritic and B-cells, and CNS cells, including neurons and glial cells. Most patients suffer from a relapsing-remitting disease course that is characterized by bouts of inflammation and neurodegeneration, which eventually transitions into progressive MS (Dobson and Giovannoni, 2019). Yet, the precise molecular causes underlying MS as well as the mechanisms driving either relapsing-remitting or progressive disease progression, remain largely unknown. There is no cure for MS and current treatments are mainly focused on the relapsing-remitting phase of the disease and they primarily target the immune system.

In this review, the function of autophagy in regulating neuroinflammation and neurodegeneration in MS is discussed, with a particular focus on how autophagy interferes with the regulation and functioning of different cell types that contribute to the pathophysiology of this devastating disease.

THE REGULATION AND MECHANISM OF AUTOPHAGY

Different types of autophagy have been described based on their differences in regulation, type of cargo, and the lysosomal delivery mechanism: chaperone-mediated autophagy, microautophagy, and macroautophagy (Feng et al., 2018). These processes are described in detail elsewhere (Martinez-Vicente and Cuervo, 2007; Cuervo, 2010; Li et al., 2012; Feng et al., 2014) and here we focus on the regulation of macroautophagy since this process is best described in brain disorders (Nixon, 2013; Liang and Le, 2015; Menzies et al., 2017; van Beek et al., 2018; Yin et al., 2018; Levine and Kroemer, 2019; Stamatakou et al., 2020).

Macroautophagy, hereafter referred to as autophagy, is characterized by the sequestration of cytoplasmic substrates by double-membrane vesicles called autophagosomes, which originates from membranous cisterna, the phagophores, generated *de novo* upon autophagy induction. Completed autophagosomes then fuse with lysosomes to deliver their cargo in the interior of this hydrolytic organelle. The metabolites resulting from the degradation of the autophagosomal cargoes are recycled back to the cytosol for the synthesis of new proteins or are used for the generation of energy (Lahiri et al., 2019).

Autophagy is a highly conserved and dynamic process that can be subdivided into five sequential steps; (i) induction and nucleation of the phagophore, (ii) phagophore elongation, (iii) phagophore closure and autophagosome maturation, (iv) autophagosome fusion, and (v) cargo degradation (**Figure 1**). These steps involve a cascade of events that are mediated by proteins, most of which have been named as autophagy-related (ATG) proteins (**Figure 1**; Nakatogawa, 2020). Upon autophagy induction, the ULK kinase complex, which consists of the serine/threonine kinases ULK1 or ULK2, FIP200, ATG13, and ATG101, gets activated through self-phosphorylation and stimulates the formation of the class III phosphatidylinositol 3-kinase (PI3KC3) complex (Nakatogawa, 2020). The PI3KC3 complex consists of the BECLIN1, VPS34, VPS15, ATG14, and NRBF2 subunits, and generates phosphatidylinositol 3-phosphate (PI3P) on the phagophore membrane (Nakatogawa, 2020). PI3P is key for the recruitment of several downstream ATG proteins that bind to this lipid, such as WIPI2 (Qian et al., 2017). Together with the ULK complex and ATG9A-positive vesicles, PI3KC3 catalyzes the nucleation of the phagophore (**Figure 1**; Nakatogawa, 2020). The elongation process involves two ubiquitin (Ub)-like conjugation systems that is composed by several ATG proteins. The first system involves the activation of ATG12 by ATG7 which is then transferred via ATG10 to ATG5 to generate the ATG12-ATG5 conjugate, which associates to ATG16L1. This is then recruited to the phagophore membrane by WIPI2, forming a multimeric complex (**Figure 1**; Nakatogawa, 2020). In parallel, ATG7, ATG4, and ATG3 are involved in another system that is responsible for the conjugation of LC3 proteins to phosphatidylethanolamine (PE). This conjugation occurs on the phagophore membrane and is guided by the ATG12-ATG5-ATG16L1 complex (**Figure 1**; Nakatogawa, 2020). Conjugated LC3 proteins are present on the internal and external surface of the expanding phagophore to mediate the expansion and closure of the autophagosome (Nakatogawa, 2020). Once autophagosomes are completed, they traffic toward lysosomes and fuse with these organelles through an event mediated by SNARE proteins and other fusion co-factors, to form the so-called autolysosomes (**Figure 1**). After fusion, the content of the autophagosome is exposed to lysosomal enzymes and the metabolites generated by degradation are recycled to the cytosol via permeases on the limiting membrane of lysosomes (Lahiri et al., 2019).

Autophagy can either be non-selective, referred to as bulk autophagy and it is activated, e.g., under starved conditions to recycle cellular components in an apparent random manner, or selective. During selective types of autophagy, damaged or superfluous organelles but also other structures, including mitochondria (mitophagy), lipid droplets (lipophagy), ribosomes (ribophagy), and invading pathogens (xenophagy), are specifically and exclusively sequestered by autophagosomes (Kirkin and Rogov, 2019). The pool of PE-conjugated LC3 proteins in the inner surface of phagophores promote the cargo engulfment via LC3-interacting regions (LIR) that are present on the so-called autophagy receptors, some of which are soluble (e.g., p62/SQSTM1, NDP52, or OPTN) and bind to ubiquitinated cargo, while other are present on organelles (e.g., NIX on mitochondria or FAM134B on the endoplasmic reticulum) (**Figure 1**; Kirkin and Rogov, 2019). This recognition system allows selective degradation of specific cargo.

Under nutrient-rich conditions, autophagy is negatively regulated by the mammalian target of rapamycin complex 1 (mTORC1) that phosphorylates and inactivates the ULK kinase complex (**Figure 1**; He and Klionsky, 2009; Zachari and Ganley, 2017). Upon removal of nutrients or energy, autophagy is induced via inhibition of mTORC1 and/or through direct phosphorylation and activation of the ULK kinase complex by

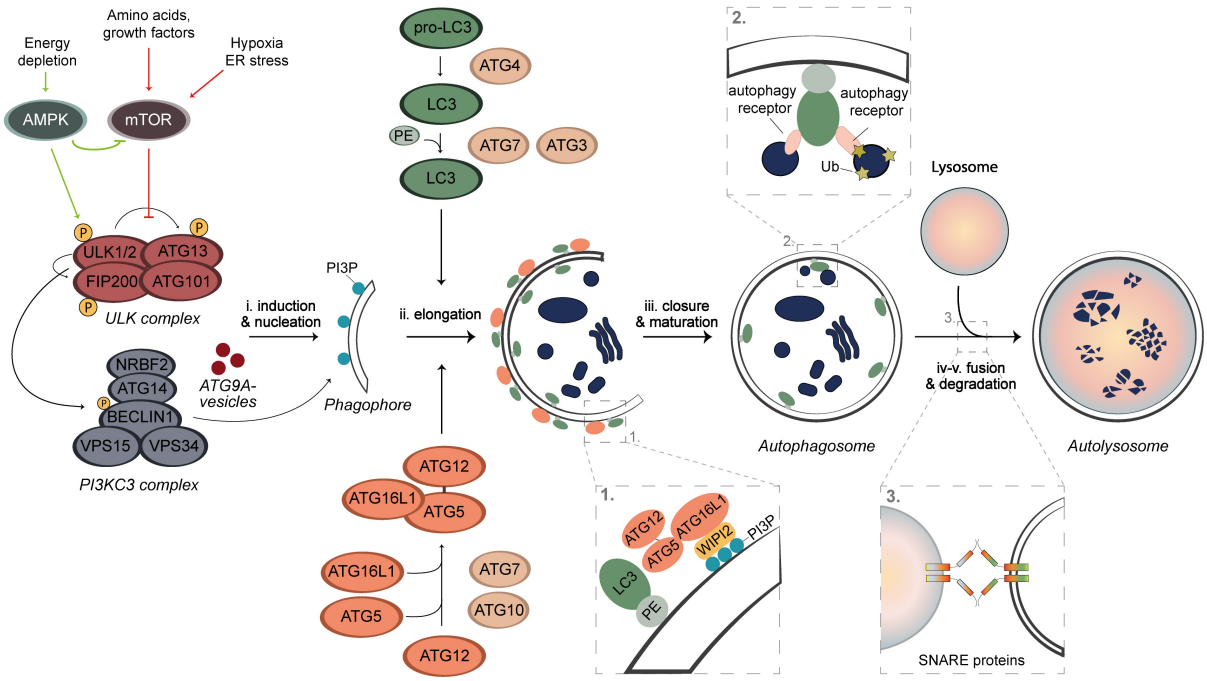

FIGURE 1 | Schematic overview of autophagy. Under nutrient rich conditions, the autophagy process is negatively regulated by mTORC1, whose activity can be inhibited through AMPK activation, starvation, hypoxia, or stress. The latter lead to a de-repression of the ULK kinase complex, which self-activates through autophosphorylation and stimulates the recruitment and activation of the PI3KC3 complex. PI3KC3 produces PI3P on the phagophore membrane, which is needed for the recruitment of the ubiquitin complexes to the membrane of the autophagosome. The nucleation is mediated by ATG9A vesicles, ULK, and PI3KC3 complexes. The autophagosome formation requires two Ub-like conjugation systems. LC3 proteins are post-translationally processed by ATG4 proteases and upon induction of autophagy, they are activated by ATG7 and ATG3 enzymes and conjugated with PE. This event is guided by a complex formed by the second Ub-like conjugation system. ATG7 activates ATG12, which is then covalently linked to ATG5 by ATG10 and subsequently associates with ATG16L1 to form a multimeric complex. This complex is anchored onto the phagophore by interacting with the PI3P effector protein WIPI2. The proteins on the external surface dissociate after completion, whereas LC3-PE permanently integrates on the internal surface of the membrane. The complete autophagosome ultimately fuses with lysosomes in a SNARE-mediated manner to form an autolysosome, in which the autophagosomal cargo is degraded by lysosomal enzymes.

adenosine monophosphate-activated protein kinase (AMPK), resulting in the activation of the downstream machinery of autophagy and consequently initiating this process (**Figure 1**; He and Klionsky, 2009; Puente et al., 2016; Keller and Lünemann, 2017; Zachari and Ganley, 2017). The modulation of these kinases is currently the major strategy to induce autophagy *in vivo* and in patients (Menzies et al., 2017; Djajadikerta et al., 2020).

AUTOPHAGY AND NEURODEGENERATION

A hallmark shared by many neurodegenerative diseases of the CNS is neuronal loss, which can have a range of causes, from the formation of cytotoxic aggregates to mitochondrial dysfunction and/or iron accumulation (Wong and Holzbaur, 2014).

Neurons heavily depend on autophagy for their survival and maintenance of homeostasis (Hara et al., 2006; Komatsu et al., 2006), and therefore it is not surprising that dysfunction of this process causes neurodegenerative diseases (Fujikake et al., 2018). Defects in different steps of the autophagy process, such as impaired autophagosome formation, inhibited autolysosome formation, or disrupted lysosomal function have been observed, e.g., Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD) (Nixon, 2013; Menzies et al., 2017).

Consistently, loss of *Atg7* or *Atg5* in the CNS of mice or neurons causes neurological defects and severe damage to neurons (Komatsu et al., 2006; Cuervo, 2010; Nixon, 2013; Stavoe and Holzbaur, 2019). Autophagy is important to degrade physiological and potentially cytotoxic protein aggregates and has a protective effect against the disease-associated aggregates characterizing AD, HD, and PD (Ravikumar et al., 2004; Nixon and Yang, 2011; Nixon, 2013; Menzies et al., 2017; Fujikake et al., 2018). The conditional deletion of *ATG* genes in mice leads to the accumulation of aggregates (Hara et al., 2006; Komatsu et al., 2006) and progressive neuronal death in different areas of the brain (Menzies et al., 2017). In MS lesions, extracellular aggregates of fibronectin are observed (Stoffels et al., 2013), however, it remains to be determined whether their appearance is connected to a deficient ATG machinery. In addition to aggregate removal, autophagy can degrade damaged mitochondria, which when impaired, can also contribute to neuronal damage and death (Wong and Holzbaur, 2014). Consequently, pharmacological induction of autophagy showed beneficial effects in a wide range of neurodegenerative diseases, such as HD and AD (Menzies et al., 2017).

Besides the intracellular defects in neurons that lead to neuronal damage, external stimuli can also cause neuronal loss. For example, neuroinflammation is often observed in neurodegenerative diseases, where it contributes to neuronal damage (Rubinsztein et al., 2015), and autophagy is emerging as an important modulator of inflammation (discussed below). Moreover, autophagy is critical for debris clearance, and its impairment delays myelin debris clearance after nerve injury (Jang et al., 2016), which prevents efficient remyelination and further leads to neuronal damage and neurodegeneration, which are typical in MS.

AUTOPHAGY AND INFLAMMATION

The immune system is essential to maintain systemic health by eliminating pathogens and preventing infections, and damaged cells. The inflammatory response of immune cells plays an essential role in this process and involves many cell types. Autophagy has been implicated in both the innate and adaptive immune response, playing a role in pathogen removal, antigen presentation, cytokine production, lymphocyte survival, and development of specific cell types (Miller et al., 2008; Levine et al., 2011; Shi et al., 2012; Deretic et al., 2013; Qian et al., 2017; Yin et al., 2018). The link between autophagy and inflammation is complex and reciprocal since they can either induce or suppress each other through different mechanisms (Levine et al., 2011; Deretic et al., 2013; Liang and Le, 2015). Therefore, it is not surprising that autophagy has been functionally and/or pathologically connected to several neuroinflammatory diseases, including AD, HD, amyotrophic lateral sclerosis (ALS), and MS (Levine et al., 2011; Muller et al., 2017; Yin et al., 2018).

Autophagy can be induced by different pro-inflammatory stimuli, such as toll-like receptor (TLR) activation, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs) (Harris and Keane, 2010; Levine et al., 2011; François et al., 2014; Liang and Le, 2015; Yin et al., 2018). On the other hand, it can be inhibited by Th2-associated pro-inflammatory cytokines, such as IL-4 and IL-13 (Harris et al., 2007; Harris and Keane, 2010; Park et al., 2011; Deretic et al., 2013). In its turn, autophagy inhibits, for example, the inflammatory IL-1β and IL-18 responses (Shi et al., 2012; Liang and Le, 2015; Zhang H. et al., 2016) by degrading inflammasomes (Shi et al., 2012; Deretic et al., 2013). Further, it also prevents the production of reactive oxygen species (ROS) that activate inflammasomes by eliminating damaged mitochondria (Qian et al., 2017). Overall, autophagy is a negative feedback regulator of the immune system, participating in the resolution of inflammation and returning it to homeostasis (Levine et al., 2011). However, autophagy is also implicated in T-cell survival and polarization, the differentiation and survival of antibody-secreting plasma cells, and the enhancement of antigen presentation in dendritic cells (DCs) (Pengo et al., 2013; Conway et al., 2013; Deretic et al., 2013; Qian et al., 2017), which are all processes that form the core of immune responses. Thus, dysregulation of autophagy can prolong and make persisting inflammatory responses after an insult, possibly leading to autoimmune and inflammatory diseases.

Genome-wide association studies have revealed the connection of several *ATG* genes with inflammatory and autoimmune disorders (Muller et al., 2017). It is important to note that the regulation of autophagy varies in different inflammatory diseases. Pharmacological inducers of autophagy appear to be protective against psoriasis (Varshney and Saini, 2018) and inflammatory bowel disease (Saitoh et al., 2008), whereas inhibition of this process ameliorates illnesses such as systemic lupus erythematosus (Clarke et al., 2015), rheumatoid arthritis (Lin et al., 2013), and MS (Kovacs et al., 2012).

The crosstalk between autophagy and the immune system emphasizes the importance of this process in the pathogenesis of autoimmune disorders, including MS.

AUTOPHAGY AND MS

Multiple sclerosis is characterized by inflammation, demyelination, and neurodegeneration, all processes that have been connected to autophagy, and therefore, investigating autophagy in the context of MS is relevant. In blood samples from MS patients, several *ATG* genes involved in multiple steps of the autophagy process were differently expressed; *ATG9A* and *BECN1* were downregulated, while *ULK1, ULK2,* and *ATG5* were upregulated (Igci et al., 2016). In addition, in experimental autoimmune encephalomyelitis (EAE), an MS mouse model, LC3 and BECLIN1 protein levels were reduced while those of p62/SQSTM1 were increased in the spinal cords of these animals. Moreover, inhibition of mTORC1 ameliorated disease severity (Boyao et al., 2019), suggesting that autophagy is negatively affected in EAE mice. Inhibition of autophagy can also result in the accumulation of damaged mitochondria and the production of ROS (Chen et al., 2008; Hassanpour et al., 2020), which both contribute to the demyelination process in MS. Another approach to enhance autophagy is through caloric restriction, where cycles of a fasting-mimicking diet are applied, and this regime has been shown to ameliorate disease severity and stimulates remyelination in both EAE mice and relapsing-remitting MS patients (Choi et al., 2016).

Importantly, a few studies have indicated that autophagy is differently involved in both relapsing and progressive forms of MS. In a cohort study, autophagic activity was increased in relapsing-remitting MS patients (Hassanpour et al., 2020), and ultrastructural analyses revealed the presence of synaptic vesicle-containing autophagosomes in the dentate nucleus from a chronic MS patient (Albert et al., 2017), suggesting a pathological role of autophagy in MS. Treatment with an mTORC1 inhibitor, however, resulted in beneficial effects in both relapsing-remitting EAE mice (Esposito et al., 2010) and patients with MS (Hassanpour et al., 2020). This emphasizes the importance to further elucidate how autophagy is involved in different forms of MS.

Although autophagy is important to maintain homeostasis in all cell types, its requirement for other functions and consequently its regulation varies in the different cell types and

consequently its regulation differs as well (Liang and Le, 2015). This aspect also emerges in the context of MS, in which autophagy appears to contribute to the pathology in DCs, T-cells and B-cells, while it has a protective role in neurons and glial cells.

Dendritic Cells

DCs are the main peripheral antigen-presenting cells (APCs) that can trigger a T-cell response (Nuyts et al., 2013). Antigen presentation is required for both T-cell development and their activation, through the expression of surface molecules and cytokine secretion from DCs (Yogev et al., 2012). DCs are the most efficient APCs for reactivating myelin-specific $CD4^+$ T-cells in the CNS (Yogev et al., 2012; Mohammad et al., 2013), and they are present in cerebrospinal fluid (CSF) and CNS lesions of MS patients (Nuyts et al., 2013).

It was hypothesized that removing DCs could inhibit EAE development, however, depletion of DCs in mice showed a stronger inflammatory response and enhanced EAE severity (Yogev et al., 2012). The levels of regulatory T-cells (Treg) were also lower (Yogev et al., 2012; Mohammad et al., 2013), confirming the important role of DCs in regulating T-cell homeostasis. In addition, a study where major histocompatibility complex (MHC) class II expression was only restricted to DCs, revealed that DCs are sufficient to present antigens to T-cells in order to mediate CNS inflammation in EAE mice (Greter et al., 2005). Altogether, these data show that the status of DCs is crucial for MS development, i.e., steady-state DCs play a protective role by inducing self-tolerance and by differentiating Treg cells, whereas activated DCs are responsible for the stronger immunogenic response by activating $CD4^+$ T-cells (Greter et al., 2005; Yogev et al., 2012; Mohammad et al., 2013). These observations have raised the question whether the molecular pathway of antigen presentation to $CD4^+$ T-cells could be modulated to prevent immune activation.

DCs phagocytose antigens and after their processing, the resulting peptides are presented on MHC class I and II molecules on the cell surface to activate $CD8^+$ and $CD4^+$ T-cells, respectively. During immune activation, autophagy is involved in host protection by delivering cytoplasmic antigens to lysosomes for subsequent presentation on MHC class II (Paludan et al., 2005; Bhattacharya et al., 2014; Yang et al., 2015; Schmid et al., 2007). In addition, extracellular compounds are degraded by LC3-associated phagocytosis (LAP), which depends on several ATG proteins (Lai and Devenish, 2012). This suggests that the ATG machinery might be involved in the myelin peptide presentation on MHC class II molecules and subsequently activation of $CD4^+$ autoreactive T-cells (**Figure 2a**). Studies supporting this hypothesis showed that DCs lacking *Atg5* or *Atg7* reduced the incidence and severity of EAE (Bhattacharya et al., 2014; Keller et al., 2017; Hassanpour et al., 2020). The absence of ATG proteins in DCs caused a reduction of myelin peptide presentation and less activated $CD4^+$ T-cells during EAE, however, it did not affect the levels of $CD8^+$ T-cells (Bhattacharya et al., 2014; Keller et al., 2017; Hassanpour et al., 2020). Interestingly, autophagy-deficient DCs completely inhibited the development of EAE via adoptive transfer of primed encephalitogenic T-cells (Keller et al., 2017), suggesting that ATG proteins are important for the activation of primed myelin-specific $CD4^+$ T-cells. Moreover, deletion of *ATG* genes in DCs did not affect other functions of DCs (Lee et al., 2010; Bhattacharya et al., 2014; Keller et al., 2017), indicating their specific importance for antigen presentation. Pharmacological inhibition of autophagy with chloroquine before EAE onset delayed disease progression and reduced EAE severity when administered during EAE development (Bhattacharya et al., 2014). However, this approach is not specific for autophagy and also affects LAP as well as other processes relying on lysosomal proteolytic activity. Therefore, further investigation is necessary to reveal whether autophagy is involved in antigen presentation of myelin-derived peptides in DCs or whether this is regulated by ATG protein-dependent phagocytic processes.

Altogether, DCs have both protective and pathological roles in MS, and autophagy could be important for the $CD4^+$ T-cell-mediated autoimmune responses, thereby contributing to the pathological traits of DCs in MS.

T-Cells

T-cells originate from bone marrow-derived hematopoietic stem cells. Lymphoid precursor cells migrate via the blood to the thymus where they develop into mature T-lymphocytes (Jia and He, 2011; Parekh et al., 2013; Bronietzki et al., 2015). T-cells are part of the adaptive immune system and are important players in both the development and modulation of inflammation. It is generally accepted that autoreactive T-cells against myelin in the CNS are key contributors to MS pathology (Group Nature Publishing, 2001; Glass et al., 2010; Liang and Le, 2015). The current notion is that T-cells are activated in the periphery by APCs, in particular by DCs, and differentiate into autoreactive T-cells. These autoreactive T-cells enter the CNS by damaging the blood-brain barrier, and in the CNS, they get reactivated and amplified (Group Nature Publishing, 2001; Glass et al., 2010; Chihara, 2018), and attack myelin sheaths of axons, resulting in denuded axons and ultimately in neuronal loss (Group Nature Publishing, 2001; Glass et al., 2010). Although, MS is thought to be a $CD4^+$ T-cell-mediated autoimmune disease, an increasing number of studies has reported a role of $CD8^+$ T-cells in the initial relapse phase of MS since the frequency of CD8+ T-cells appearance in lesions was increased (Friese and Fugger, 2009; Salou et al., 2015). Several studies also highlighted the importance of T-cells in MS pathology; they showed that the balance between $CD4^+$ T-cells, $CD8^+$ T-cells, and Tregs is disturbed (Fletcher et al., 2010; Chihara, 2018). This might be due to higher levels of autoreactive T-cells that showed increased proliferation and prolonged survival in MS patients (Sawcer et al., 2011; Igci et al., 2016).

During the past decades, autophagy has been implicated in various biological processes of T-cells, such as maintenance of T-cell homeostasis, differentiation, and activation (Li et al., 2006; Pua and He, 2007; Pua et al., 2007, 2009; Botbol et al., 2016; Paunovic et al., 2018; Macian, 2019). The expression levels of the *ATG5* gene in T-cells from MS patients are increased in blood and brain sections (Alirezaei et al., 2009; Yang et al., 2015), indicating a possible involvement of autophagy in the activation of autoreactive T-cells. Consistently, autophagosomes were only

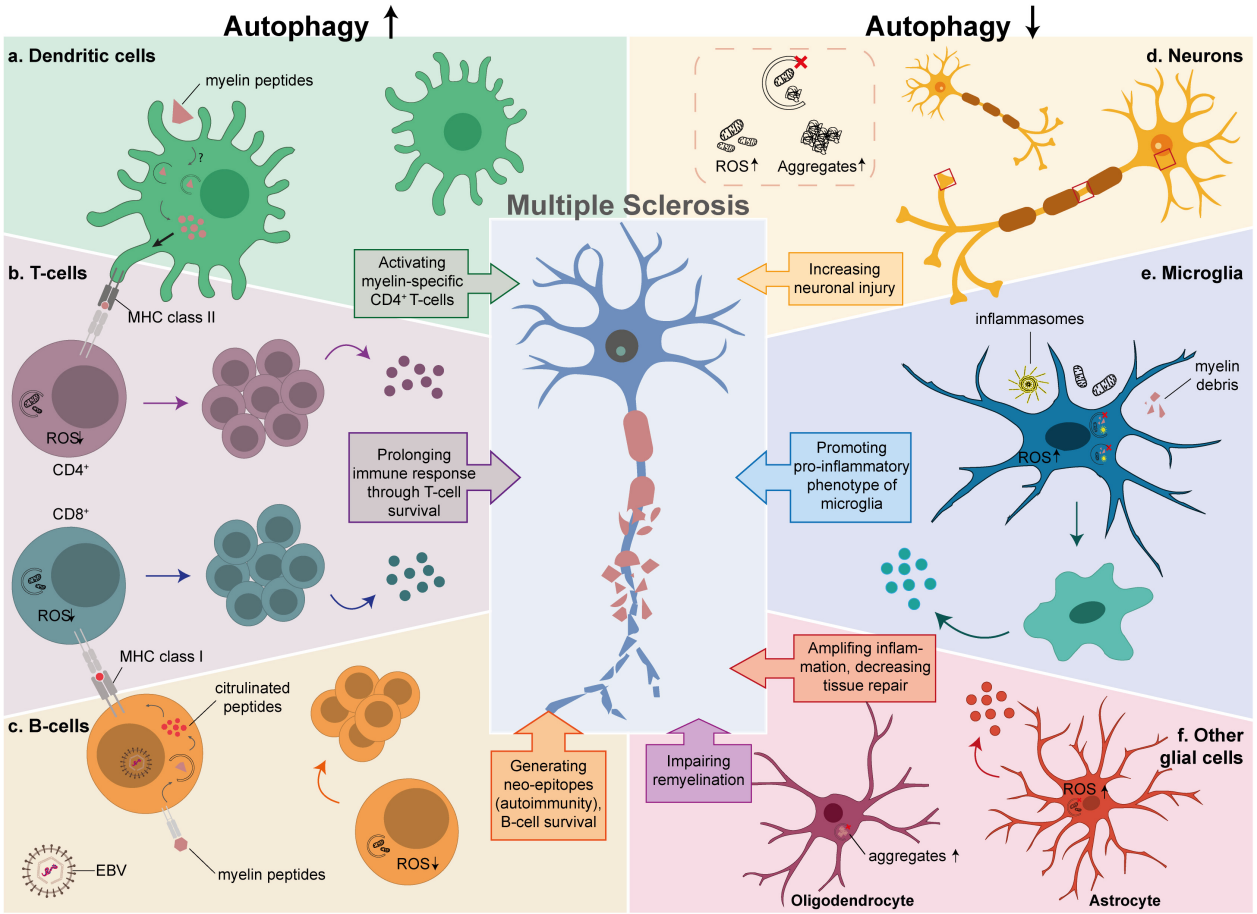

FIGURE 2 | The possible link between autophagy and MS in different cell types. Autophagy is critical in the development and function of cells that play an important role in MS pathology. The left side of the diagram shows the effects of an enhancement of autophagy in T-cells, B-cells and DCs, while the right side depicts the effects of autophagy downregulation in neurons, microglia and other glial cells. **(a)** Increased myelin processing and antigen presentation to CD4+ autoreactive T-cells in DCs by either autophagy or LAP. **(b)** Prolonged survival of activated CD4+ and CD8+ T-cells due to low levels of ROS, resulting in proliferation and secretion of pro-inflammatory cytokines. **(c)** Productive processing of antigens in EBV-infected B-cells, that results in citrullinated peptides that are presented as neo-epitopes to CD8+ T-cells, and prolonged survival of B-cells by degrading damaged mitochondria. **(d)** Defective autophagy in neurons results in increased ROS levels and aggregate formation. **(e)** Insufficient clearance of damaged mitochondria, inflammasomes, and myelin debris in microglia, promotes a pro-inflammatory phenotype caused by autophagy or LAP. **(f)** Decreased tissue repair and secretion of pro-inflammatory cytokines by astrocytes (red), aggravates the inflammatory response and impaired remyelination by OLs (purple).

detected in $CD4^+$ T-cells after T-cell receptor activation, and not in resting, naïve cells (Li et al., 2006; Hubbard et al., 2010; Macian, 2019). Mice experiments with either *Atg5*- or *Atg7*-deficient $CD4^+$ and $CD8^+$ T-cells showed indeed multiple defects, including reduced survival and a defect in T-cell proliferation in response to antigen stimulation (Pua et al., 2007; Alirezaei et al., 2009; Keller and Lünemann, 2017; Paunovic et al., 2018; Yin et al., 2018). *Beclin1*-deficient $CD4^+$ T-cells prevented EAE development in mice, and T-cells were absent in the CNS (Alirezaei et al., 2009; Kovacs et al., 2012; Yin et al., 2018).

It has also been suggested that autophagy regulates cell death in activated T-cells (Kovacs et al., 2012). *Beclin1*-deficient $CD4^+$ T-cells are more susceptible to apoptotic stimuli since they accumulate cell death-related proteins, such as procaspase-3, procaspase-8, and BCL2-interacting mediator (BIM). In particular, cell death-related proteins have been found in autophagosomes, and these proteins accumulated in autophagy-deficient T-cells (Li et al., 2006; Pua and He, 2007; Trapp and Nave, 2008; Pua et al., 2009; Kovacs et al., 2012; Salminen et al., 2013). This suggests a pro-survival function of autophagy in activated T-cells through the turnover of cell death-related proteins, which then will prolong their survival and consequent rapid amplification in the CNS that will initiate a persisting immune response (**Figure 2b**; Botbol et al., 2016). In addition, other reports have revealed that organelle turnover in T-cells critically depends on autophagy. Specifically, ER and dysfunctional mitochondria accumulate in T-cells when autophagy is blocked, which in turn leads to an increase in ROS levels and consequent cell death (Pua et al., 2009; Hubbard et al., 2010; Jia and He, 2011; Kovacs et al., 2012; Macian, 2019). An important finding is that subtypes of T-cells such as Th17 and Th1 are not equally susceptible to cell death after *Beclin1*-deletion

(Kovacs et al., 2012), which might be due to the importance of autophagy in cell survival in different subsets of T-cells, or other roles of Beclin1 outside the context of autophagy.

These findings show that enhanced autophagy promotes T-lymphocyte survival and proliferation, thereby positively contributing to MS pathogenesis.

B-Cells

B-cells play an important role in immune processes by generating antibodies that are directed to pathogens (Lehmann Horn et al., 2013; Li et al., 2018). Moreover, B-cells are recognized as APCs and thereby contribute to the regulation of immune processes (Hirotani et al., 2010; von Büdingen et al., 2015; Arneth, 2019). The crucial role of B-cells in MS pathology became clear when depletion of B-cells in MS patients with anti-CD20 antibodies led to the suppression of an inflammatory response, reducing the formation of new lesions and disease progression (Hauser et al., 2008; Gelfand et al., 2017; Mulero et al., 2018; Sospedra, 2018; Arneth, 2019). Similar to DCs and T-cells, B-cells consist of different subpopulations. B-cells in MS patients show increased secretion of pro-inflammatory cytokines (Bar-Or et al., 2010) and a deficiency in IL-10 production (Duddy et al., 2007), suggesting a perturbed balance between pro-inflammatory and regulatory B-cells, respectively. It is not fully understood how these B-cells contribute to MS pathology.

One environmental risk factor that has been linked to MS is the Epstein–Barr virus (EBV) (Sospedra, 2018). EBV infects B-cells, which in turn cross-present autoantigens that can activate T-cells against myelin (Bar-Or et al., 2020). The link between EBV and MS development is quite strong since nearly all MS patients had a past EBV infection (Ascherio and Munger, 2010; Guan et al., 2019). It appears that EBV infection during adolescence is a prerequisite to develop MS, although not sufficient on its own (Ascherio and Munger, 2010; Guan et al., 2019; Bar-Or et al., 2020). B-cells from MS patients show an increased expression of APC-related markers (Sospedra, 2018; Guan et al., 2019) and experiments in EAE mice uncovered that EBV upregulates antigen cross-presentation of infected B-cells to $CD8^+$ T-cells (Dunham et al., 2017; Jakimovski et al., 2017). These results indicate that EBV influences the antigen presentation of B-cells. This notion is also supported by EAE animal experiments, where uninfected B-cells prevented autoimmunity by degrading self-antigens, while these antigens, which are generated by the productive processing of myelin oligodendrocyte glycoprotein (MOG), are presented to autoreactive T-cells in EBV infected B-cells, thereby inducing an immune activity (Thorley-Lawson and Mann, 1985; Livingston et al., 1997; Jagessar et al., 2016; Dunham et al., 2017; Jakimovski et al., 2017; Morandi et al., 2017; Guan et al., 2019).

It has been suggested that the productive processing of antigens results from the citrullination of peptides, and this is enhanced by an EBV infection ('t Hart et al., 2016; Morandi et al., 2017; Bar-Or et al., 2020). Citrullination is a posttranslational modification that converts arginine into citrulline, this conversion is relevant for antigen presentation because it generates neo-epitopes that can be recognized by the immune system (Guan et al., 2019). Autophagy is responsible for the generation and processing of citrullinated peptides (Ireland and Unanue, 2011; Münz, 2016; Morandi et al., 2017), resulting in neo-epitopes that could be recognized by T-cells and induce an autoimmune response (Alghamdi et al., 2019). An interesting finding has been that the processing of citrullinated peptides depends on autophagy induction in B-cells, whereas unmodified peptides are unaffected when autophagy was blocked in this cell type with 3-methyladenine (Ireland and Unanue, 2011). In particular, citrullination of the MOG peptide at Arg46 protected this peptide from degradation in EBV-infected B-cells. Interestingly, Arg46 in MOG is positioned within the LIR motif that is important for its selective targeting by autophagy (Birgisdottir et al., 2013; Morandi et al., 2017). These findings suggest a mechanistic link between EBV, autophagy, and autoimmunity. EBV-infected B-cells indeed display more autophagosomes, and MOG peptides are present inside these vesicles (Ireland and Unanue, 2011; Morandi et al., 2017). Moreover, pharmacological induction of autophagy with rapamycin further enhanced the protection of citrullinated MOG peptides from degradation (Camilli et al., 2016; Morandi et al., 2017), indicating that this pathway protects myelin peptides against destructive processing and consequently promotes their presentation to T-cells. Altogether, EBV infection in B-cells is responsible for inducing autophagy, which is important for altering antigens that can initiate autoimmunity against myelin in MS (**Figure 2c**).

In addition to the role in the generation and processing of citrullinated peptides in EBV-infected B-cells, autophagy is also important for B-cell survival, development, and activation (Rathmell, 2012; Puleston and Simon, 2014; Bhattacharya and Eissa, 2015), similarly to what happens in T-cells (see section "T-Cells"). Thus, like DCs and T-cells, autophagy activation in B-cells appears to contribute to the pathogenicity of MS rather than to its prevention.

Neurons

Currently, axonal damage is considered part of a secondary phase of MS, which is caused by an initial inflammation in the periphery that is subsequently followed by demyelination in the CNS (Ferguson et al., 1997; Trapp et al., 1998; Tsunoda and Fujinami, 2002). This concept is known as the outside-in model. However, this model is debated questioning whether the axonal injury is exclusively caused by an immune response initiated in the periphery or directly from the neurons. Moreover, it cannot be excluded that neuronal loss is the primary phase of MS, which is then followed by a second phase characterized by demyelination and an inflammation response (Lovas et al., 2000; Bjartmar et al., 2001; Tsunoda and Fujinami, 2002). This scenario is known as the inside-out model. Infections in neurons can indeed induce neuronal damage, which leads to demyelination and neurodegeneration (Tsunoda et al., 2003), and these observations support the inside-out model. However, there are also examples from experiments with animal models of MS that showed evidence of axonal injury without any signs of demyelination (Ferguson et al., 1997; Trapp et al., 1998; Tsunoda et al., 2003). Thus, it is possible that in addition to demyelination,

other triggers are involved in the induction of neuronal loss during MS (Tsunoda and Fujinami, 2002).

Neurons depend on autophagy for clearing misfolded or aggregated proteins and damaged organelles, and autophagy is continuously active at basal levels in neuronal cells under normal conditions (Hara et al., 2006; Plaza-Zabala et al., 2017; Feng et al., 2018; Stavoe and Holzbaur, 2019). Autophagy is active in each neuronal compartment, however, the axons and dendrites are the most metabolically demanding regions where autophagy is crucial (Stavoe and Holzbaur, 2019). It is known that basal autophagy in neurons is essential for protein quality control, pruning, development, and neuronal survival (Hara et al., 2006; Komatsu et al., 2006; Wong and Cuervo, 2010; Feng et al., 2017; Plaza-Zabala et al., 2017; Stavoe and Holzbaur, 2019). Defects in neuronal autophagy results in aggregate formation and neuronal damage, which ultimately leads to neuronal death (Hara et al., 2006; Komatsu et al., 2006, 2007; Liang and Le, 2015; Feng et al., 2017; Stavoe and Holzbaur, 2019; **Figure 2d**). Defective autophagy has been observed in the spinal cords of EAE mice, and pharmacological induction of autophagy with rapamycin reduced demyelination, inflammation, and neuronal loss (Feng et al., 2017, 2018). In contrast, inhibition of autophagy non-specifically with 3-methyladenine, resulted in higher neuronal apoptosis in EAE mice (Feng et al., 2017), suggesting that autophagy dysfunction could be associated with EAE-induced neuronal loss. Another study showed that LC3 protein expression levels in neurons were higher in control mice compared to EAE mice (Feng et al., 2018), however, this could indicate that autophagy is either reduced or enhanced in neurons during EAE development. Future research has to reveal whether neuronal autophagy contributes to the neurobiological and neuropathological features of MS.

Microglia

Microglia are the tissue-resident macrophages of the CNS and they form the first line of defense in the CNS (Schulz et al., 2012; Kierdorf et al., 2013; Luo et al., 2017). Microglia get activated upon tissue injury or a stimulus via a variety of cell surface receptors (Augusto-Oliveira et al., 2019). Activated microglia are essential for inflammatory responses in the CNS (Ponomarev et al., 2005; Luo et al., 2017), where they are involved in phagocytosis, antigen presentation, and cytokine production (Benveniste, 1997). Microglia activation can result in either neurotoxic or neuroprotective effects, depending on the stimulus (Orihuela et al., 2016).

Activated microglia are present in CNS lesions of MS patients and animal models, and are found to be an important source of ROS and nitric oxide (NO) radicals (Gray et al., 2008; Zeis et al., 2009). Interestingly, genes identified to be associated with MS susceptibility are enriched in microglia compared to other CNS cell types (Patsopoulos et al., 2019; Guerrero and Sicotte, 2020), placing these cells in the spotlight of the disease. Nowadays, microglia are recognized as one of the key players in MS pathophysiology. However, the role of microglia in MS is complex and controversial. Microglia are heterogeneous cells that can adopt a range of different phenotypes, with different functions, in response to different stimuli (Durafourt et al., 2012; Melief et al., 2012, 2013; Boche et al., 2013; Giunti et al., 2014). A few studies have shown that activated microglia participate in both the inflammation state and demyelination, by secreting pro-inflammatory cytokines (Prineas et al., 2001; Lassmann et al., 2007; Luo et al., 2017). Microglia-deficient EAE mice are protected against gray and white matter damage (Heppner et al., 2005), and EAE severity is reduced (Bogie et al., 2014). Inhibition of microglial activation in EAE mice also resulted in a reduction of demyelination and preserved mature oligodendrocytes (OLs) (further discussed in the next section) (Nissen et al., 2018). Additionally, microglia-deficient mice showed a reduction in myelin debris clearance, resulting in impaired remyelination (Lampron et al., 2015). Microglia promote remyelination by secreting anti-inflammatory cytokines, phagocytosing myelin debris (Prineas et al., 2001; De Groot et al., 2001; Lassmann et al., 2007; Kierdorf et al., 2013; Guerrero and Sicotte, 2020), and enhancing OLs proliferation and differentiation (Li et al., 2005; Voß et al., 2012; Miron et al., 2013; Bogie et al., 2014; Lloyd et al., 2017). Taken together, microglia are involved in different phases of MS, in which they play either a pathological or a protective role.

It has been postulated that autophagy is involved in microglia-mediated neuroinflammation since there is evidence that links autophagy to the regulation of microglial inflammation (Plaza-Zabala et al., 2017). Autophagy induction in pre-stimulated microglial cells with an inflammatory stimulus, tumor necrosis factor α (TNF-α) or lipopolysaccharide (LPS), promotes microglia toward an anti-inflammatory phenotype and suppresses pro-inflammatory genes (Shao et al., 2014; Su et al., 2016; Bussi et al., 2017; He et al., 2018; Jin et al., 2018; Hassanpour et al., 2020). Conversely, autophagy inhibition leads to opposite results, regardless of the presence of an inflammatory stimulus (Shao et al., 2014; Su et al., 2016; Bussi et al., 2017; He et al., 2018; Jin et al., 2018; Hassanpour et al., 2020). Moreover, *Atg5* knockdown in microglia, enhances neurotoxicity in microglia-neuron co-cultures (Bussi et al., 2017; He et al., 2018; Jin et al., 2018), while autophagy induction by activating cannabinoid receptor 2 prevents inflammasome activation in both EAE mice (Shao et al., 2014) and microglia cultures (Shao et al., 2014; Su et al., 2016). Together, these observations indicate that autophagy is a key process in microglia as it balances their pro- and anti-inflammatory responses (**Figure 2e**).

Besides the involvement of microglial autophagy in inflammatory responses, ATG proteins are also involved in the phagocytosis and elimination of myelin debris (Sanjuan et al., 2007), which indicates the possible involvement of LAP. As a result, defective ATG machinery in microglia could lead to an inefficient clearing of myelin debris, which in turn will cause impairment in remyelination and enhanced neuroinflammation in neurodegenerative diseases (Sanjuan et al., 2007; Meikle et al., 2008; Rangaraju et al., 2010). Altogether, these observations emphasize the importance of microglial autophagy and ATG proteins in general, in MS etiology since they negatively modulate the underlying inflammatory response and promote remyelination.

Microglia are also involved in synaptic pruning during development. However, they also play a role in the synaptic loss seen in neurodegenerative conditions, such as MS

(Ramaglia et al., 2012). This action requires complement C3 that localizes to synapses which are then recognized by complement receptors expressed by microglia (Ramaglia et al., 2012; Werneburg et al., 2020). Besides their importance in synaptic pruning, these complement molecules are also involved in microglia priming which leads to an exaggerated response to a potentially minor secondary stimulus which is also connected to MS. Interestingly, besides neuronal autophagy, autophagy in microglia has also shown to control synaptic pruning (Druart and Le Magueresse, 2019; Lieberman et al., 2019). Mice that were Atg7-deficient specifically in microglia showed increased spine density (Kim et al., 2017; Lieberman et al., 2019). One of the hypotheses is that autophagy in microglia is important for degrading the phagocytosed components by microglial cells and this could be performed by LAP, which overlaps extensively with the conventional autophagy pathway (Lieberman et al., 2019). Both neuronal and microglial autophagy are involved in synaptic development and dysfunction of this process might also be involved in synaptic loss seen in MS. Further investigation is required to reveal whether inflammation is the main cause of the pathogenesis or whether dysfunction in autophagy causes both inflammation and neurodegeneration.

Oligodendrocytes

In addition to microglia, activation of OLs are also important in neuroinflammation and are involved in the development of MS (Glass et al., 2010; Liang and Le, 2015).

Oligodendrocytes differentiate from oligodendrocyte precursor cells (OPCs) and are important for the myelination of axons in the CNS (Nave and Werner, 2014), where the extensive loss of OLs has been observed in MS lesions (Wolswijk, 2000). In MS, several processes result in the injury of both OLs and OPCs, leading to demyelination and inefficient remyelination, respectively (Chang et al., 2000; Wolswijk, 2002). Autophagy is important for the survival and differentiation of OLs, and it influences their myelinating ability (Bankston et al., 2019). The enhancement of autophagy increases the thickness of the myelin sheaths as well as the numbers of myelinated axons (Smith et al., 2013). Moreover, autophagy-deficient OLs showed a reduction in the number of myelinated axons and decreased thickness of the myelin (Bankston et al., 2019). It has been suggested that a key function of autophagy in OLs is to prevent aggregation of myelin components, allowing OLs to continue with protein and lipid synthesis to form compact myelin sheaths (**Figure 2f**; Smith et al., 2013). Dysfunction of autophagy in OLs might also play a role in the field of myelin plasticity, where it may be involved in cytoplasm decompaction and decreased numbers of myelin wraps due to lower levels of OLs that ultimately leads to demyelination in MS (Hill et al., 2018; Belgrad et al., 2020). However, the exact role of autophagy in OLs and whether the disrupted OLs protein homeostasis in MS is caused by an autophagy impairment, remain to be clarified.

Astrocytes

Another important cell type in MS are astrocytes, which supports and regulates the communication between neurons and maintains the blood-brain barrier. They also participate in CNS damage repair by secreting growth factors and extracellular matrix proteins (Joe et al., 2018). Several studies have shown that astrocytes have multiple functions in the formation of MS lesions, where they can be activated during the inflammatory process and release inflammatory mediators that aggravate brain lesions (Cotrina and Nedergaard, 2002; Cornejo et al., 2018; Ponath et al., 2018; Cohen and Torres, 2019; Cressatti et al., 2019). They can also recruit peripheral immune cells to the inflammation site of the CNS (Rezai-Zadeh et al., 2009; Chompre et al., 2013; Clarke et al., 2018). Astrocytes are also involved in the repair of lesions, restricting the inflammatory damage (Sofroniew and Vinters, 2010; Cho et al., 2014). Genetic astrocyte ablation in MS mouse models aggravated tissue damage and clinical impairment by both preventing the recruitment of microglia to clear myelin debris and reducing the proliferation of OPCs (Brambilla et al., 2009, 2005; Skripuletz et al., 2013). These events result in impaired remyelination and shows the importance of astrocytes in promoting tissue repair.

Autophagy in astrocytes is important for their differentiation and maturation (Wang et al., 2014; Wang and Xu, 2020), and it is implicated in the role of astrocytes in several neurodegenerative diseases besides MS (Wang and Xu, 2020), including PD and AD. In particular, autophagy in astrocytes is important in regulating mitochondria dynamics and preserving mitochondrial network organization during inflammation. Consequently, impairment of this process results in the generation of ROS, which in turn amplifies the pro-inflammatory response and ultimately leads to the cell death of astrocytes (Lee et al., 2009; Motori et al., 2013). Moreover, autophagy in astrocytes has also been linked to neuronal survival since its inhibition with either rapamycin or transduction with small interfering RNA against *Atg5* induces neuronal death (**Figure 2f**; Malta et al., 2012; Liu et al., 2018). Together, these results underline the important role of autophagy in astrocytes to maintain homeostasis in an inflammatory environment, which contributes to neuronal survival. Whether autophagy is dysregulated in astrocytes during MS needs to be further investigated.

DISCUSSION

Defects in autophagy contribute to MS etiology. Autophagy, however, acts as a two-edged sword during MS, having both protective and detrimental effects that are cell type-dependent. As highlighted in this review, autophagy enhancement in cell types like DCs, T-cells, and B-cells, is participating to the initiation of neuroinflammation seen in MS. Inhibition of autophagy in these cells could be a potential therapeutic target. Yet, autophagy also appears to be protective against the detrimental effects of the immune system in neurons and glial cells, where it prevents both aggregate and ROS formation, modulates the inflammatory response, and promotes remyelination. To connect the role of autophagy in MS to one of the paradigms in MS etiopathogenesis ("inside-out" or "outside-in") based on the current knowledge is difficult. Autophagy is involved in both inflammation and neurodegeneration processes that are seen in MS. The findings that link autophagy to the pathology of DCs, T-cells, and B-cells, could be considered as an "outside-in" event. However, the functional role of autophagy in neurons which is affected in MS

and clearance of myelin debris by glial cells could be considered as an "inside-out" event. How the autophagy process is affected in these different cell types is an important question that needs to be answered in order to have a significant input in the ongoing debate whether MS is an "inside-out" or "outside-in" event.

Thus, the available data suggest that autophagy plays an important role in the regulation of the immune response under normal conditions and in preventing the development of an autoimmune response. This raises the possibility that modulating the autophagy process in a cell type-specific manner may limit inflammatory CNS damage and demyelination over the course of MS, which in turn would protect against neuronal death. It might be possible that the involvement of ATG genes in the phagocytosis of extracellular myelin debris and other components by DCs and microglia is rather due to LAP. However, autophagy and LAP share numerous ATG proteins, and therefore it is difficult to distinguish between the two. One known difference between autophagy and LAP is the requirement of ULK kinase complex in autophagy and not in LAP (Lai and Devenish, 2012). Additionally, ultrastructural observations of the phagosome membrane might reveal the contribution and importance of these processes in MS pathology.

In the optic of future therapies, it will be important to elucidate whether autophagy modulation is beneficial in both relapsing-remitting and progressive MS patients. However, autophagy might be more therapeutically beneficial for relapsing-remitting patients since this phase includes active inflammatory demyelinating lesions, while this phenomenology is absent in chronic progressive lesions (Dutta and Trapp, 2014).

Pharmacological interventions targeting autophagy in specific cell types might help to restore the balance of the immune system, which is a promising avenue for the treatment of autoimmune disorders. Most of the current pharmacological modulators of autophagy act on signaling cascades that regulate this process (**Figure 1**), rather than specifically target autophagy itself. This could result in off-target effects, which could be avoided by giving the treatment in cycles of brief periods. On the other hand, more direct biochemical approaches to modulate autophagy such as spermidine (Morselli et al., 2011) and TAT-beclin (Shoji-Kawata et al., 2013), are promising for the treatment of MS as they are also less invasive. Moreover, caloric restriction or exercise enhances autophagy and therefore might be effective as a treatment for MS (Choi et al., 2016).

Based on the current knowledge about the involvement of autophagy in different cell types during MS, T-cells and microglia are promising targets for cell type-specific delivery of autophagy modulators (Zhang F. et al., 2016; Schmid et al., 2017; Wang et al., 2019). In this context, nanoparticles that specifically bind to particular T-cell subsets have been designed (Schmid et al., 2017), and inhibiting autophagy in $CD4^+$ and $CD8^+$ autoreactive T-cells could prevent the initial activation of the immune response seen in MS. Since prolonged inhibition of autophagy in T-cells might negatively affect T-cell homeostasis, transient therapy is desirable. In addition, autophagy inducers in nanoparticles that are specifically targeted to microglia and macrophages (Wang et al., 2019) could selectively promote both anti-inflammatory responses and dampening of the pro-inflammatory effects, which will ultimately result in beneficial effects on the inflammation resolution, clearing of myelin debris, and remyelination. However, additional research is needed to investigate whether a nanoparticle or any other approach to either block or stimulate autophagy in a cell type-specific manner can delay MS progression. Nonetheless, autophagy is an attractive and promising target for the development of new treatments for MS and future studies investigating the precise role of this pathway in the different cell types during the course of this severe disease will be key to appropriately intervene therapeutically.

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REFERENCES

Albert, M., Barrantes-Freer, A., Lohrberg, M., Antel, J. P., Prineas, J. W., Palkovits, M., et al. (2017). Synaptic pathology in the cerebellar dentate nucleus in chronic multiple sclerosis. *Brain Pathol.* 27, 737–747. doi: 10.1111/bpa.12450

Alirezaei, M., Fox, H. S., Flynn, C. T., Moore, C. S., Hebb, A. L., Frausto, R. F., et al. (2009). Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis. *Autophagy* 5, 152–158. doi: 10.4161/auto.5.2.7348

Alghamdi, M., Alasmari, D., Assiri, A., Mattar, E., Aljaddawi, A. A., Alattas, S. G., et al. (2019). An overview of the intrinsic role of citrullination in autoimmune disorders. *J. Immunol. Res.* 2019:7592851. doi: 10.1155/2019/7592851

Arneth, B. M. (2019). Impact of B cells to the pathophysiology of multiple sclerosis. *J. Neuroinflamm.* 16:128. doi: 10.1186/s12974-019-1517-1

Ascherio, A., and Munger, K. L. (2010). Epstein-Barr virus infection and multiple sclerosis: a review. *J. Neuroimmune Pharmacol.* 5, 271–277. doi: 10.1007/s11481-010-9201-3

Augusto-Oliveira, M., Arrifano, G. P., Lopes-Araújo, A., Santos-Sacramento, L., Takeda, P. Y., Anthony, D. C., et al. (2019). What do microglia really do in healthy adult brain? *Cells* 8:1293. doi: 10.3390/cells8101293

Bankston, A. N., Forston, M. D., Howard, R. M., Andres, K. R., Smith, A. E., Ohri, S. S., et al. (2019). Autophagy is essential for oligodendrocyte differentiation, survival, and proper myelination. *Glia* 67, 1745–1759. doi: 10.1002/glia.23646

Bar-Or, A., Fawaz, L., Fan, B., Darlington, P. J., Rieger, A., Ghorayeb, C., et al. (2010). Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? *Ann. Neurol.* 67, 452–461. doi: 10.1002/ana.21939

Bar-Or, A., Pender, M. P., Khanna, R., Steinman, L., Hartung, H. P., Maniar, T., et al. (2020). Epstein–Barr virus in multiple sclerosis: theory and emerging immunotherapies. *Trends Mol. Med.* 26, 296–310. doi: 10.1016/j.molmed.2019.11.003

Beau, I., Mehrpour, M., and Codogno, P. (2011). Autophagosomes and human diseases. *Int. J. Biochem. Cell Biol.* 43, 460–464. doi: 10.1016/j.biocel.2011.01.006

Belgrad, J., De Pace, R., and Fields, R. D. (2020). Autophagy in myelinating glia. *J. Neurosci.* 40, 256–266. doi: 10.1523/JNEUROSCI.1066-19.2019

Benveniste, E. N. (1997). Cytokines: influence on glial cell gene expression and function. *Chem. Immunol.* 69, 31–75. doi: 10.1159/000058653

Bhattacharya, A., and Eissa, N. T. (2015). Autophagy as a stress response pathway in the immune system. *Int. Rev. Immunol.* 34, 382–402. doi: 10.3109/08830185.2014.999156

Bhattacharya, A., Parillon, X., Zeng, S., Han, S., and Eissa, N. T. (2014). Deficiency of autophagy in dendritic cells protects against experimental autoimmune encephalomyelitis. *J. Biol. Chem.* 289, 26525–26532. doi: 10.1074/jbc.M114.575860

Birgisdottir, ÅB., Lamark, T., and Johansen, T. (2013). The LIR motif - crucial for selective autophagy. *J. Cell Sci.* 126, 3237–3247. doi: 10.1242/jcs.126128

Bjartmar, C., Kinkel, R. P., Kidd, G., Rudick, R. A., and Trapp, B. D. (2001). Axonal loss in normal-appearing white matter in a patient with acute MS. *Neurology* 57, 1248–1252. doi: 10.1212/WNL.57.7.1248

Boche, D., Perry, V. H., and Nicoll, J. A. (2013). Review: activation patterns of microglia and their identification in the human brain. *Neuropathol. Appl. Neurobiol.* 39, 3–18. doi: 10.1111/nan.12011

Bogie, J. F., Stinissen, P., and Hendriks, J. J. (2014). Macrophage subsets and microglia in multiple sclerosis. *Acta Neuropathol.* 128, 191–213. doi: 10.1007/s00401-014-1310-2

Botbol, Y., Guerrero-Ros, I., and Macian, F. (2016). Key roles of autophagy in regulating T-cell function. *Eur. J. Immunol.* 46, 1326–1334. doi: 10.1002/eji.201545955

Boyao, Y., Mengjiao, S., Caicai, B., Xiaoling, L., Zhenxing, L., and Manxia, W. (2019). Dynamic expression of autophagy-related factors in autoimmune encephalomyelitis and exploration of curcumin therapy. *J. Neuroimmunol.* 337:577067. doi: 10.1016/j.jneuroim.2019.577067

Brambilla, R., Bracchi-Ricard, V., Hu, W. H., Frydel, B., Bramwell, A., Karmally, S., et al. (2005). Inhibition of astroglial nuclear factor KB reduces inflammation and improves functional recovery after spinal cord injury. *J. Exp. Med.* 202, 145–156. doi: 10.1084/jem.20041918

Brambilla, R., Persaud, T., Hu, X., Karmally, S., Shestopalov, V. I., Dvoriantchikova, G., et al. (2009). Transgenic inhibition of astroglial NF-KB improves functional outcome in experimental autoimmune encephalomyelitis by suppressing chronic central nervous system inflammation. *J. Immunol.* 182, 2628–2640. doi: 10.4049/jimmunol.0802954

Bronietzki, A. W., Schuster, M., and Schmitz, I. (2015). Autophagy in T-cell development, activation and differentiation. *Immunol. Cell Biol.* 93, 25–34. doi: 10.1038/icb.2014.81

Bussi, C., Ramos, J., Arroyo, D., Gaviglio, E., Gallea, J., Wang, J., et al. (2017). Autophagy down regulates pro-inflammatory mediators in BV2 microglial cells and rescues both LPS and alpha-synuclein induced neuronal cell death. *Sci. Rep.* 7:43153. doi: 10.1038/srep43153

Camilli, G., Cassotta, A., Battella, S., Palmieri, G., Santoni, A., Paladini, F., et al. (2016). Regulation and trafficking of the HLA-E molecules during monocyte-macrophage differentiation. *J. Leukoc. Biol.* 99, 121–130. doi: 10.1189/jlb.1a0415-172r

Chang, A., Nishiyama, A., Peterson, J., Prineas, J., and Trapp, B. D. (2000). NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. *J. Neurosci.* 20, 6404–6412. doi: 10.1523/JNEUROSCI.20-17-06404.2000

Chen, Y., McMillan-Ward, E., Kong, J., Israels, S. J., and Gibson, S. B. (2008). Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. *Cell Death Differ.* 15, 171–182. doi: 10.1038/sj.cdd.4402233

Chihara, N. (2018). Dysregulated T cells in multiple sclerosis. *Clin. Exp. Neuroimmunol.* 9, 20–29. doi: 10.1111/cen3.12438

Cho, M. H., Cho, K., Kang, H. J., Jeon, E. Y., Kim, H. S., Kwon, H. J., et al. (2014). Autophagy in microglia degrades extracellular β-amyloid fibrils and regulates the NLRP3 inflammasome. *Autophagy* 10, 1761–1775. doi: 10.4161/auto.29647

Choi, A. M., Ryter, S. W., and Levine, B. (2013). Mechanisms of disease: autophagy in human health and disease. *N. Engl. J. Med.* 368, 651–662. doi: 10.1056/NEJMra1205406

Choi, I. Y., Piccio, L., Childress, P., Bollman, B., Ghosh, A., Brandhorst, S., et al. (2016). A diet mimicking fasting promotes regeneration and reduces autoimmunity and multiple sclerosis symptoms. *Cell Rep.* 15, 2136–2146. doi: 10.1016/j.celrep.2016.05.009

Chompre, G., Cruz, E., Maldonado, L., Rivera-Amill, V., Porter, J. T., and Noel, R. J. Jr. (2013). Astrocytic expression of HIV-1 Nef impairs spatial and recognition memory. *Neurobiol. Dis.* 49, 128–136. doi: 10.1016/j.nbd.2012.08.007

Clarke, A. J., Ellinghaus, U., Cortini, A., Stranks, A., Simon, A. K., Botto, M., et al. (2015). Autophagy is activated in systemic lupus erythematosus and required for plasmablast development. *Ann. Rheum. Dis.* 74, 912–920. doi: 10.1136/annrheumdis-2013-204343

Clarke, L. E., Liddelow, S. A., Chakraborty, C., Münch, A. E., Heiman, M., and Barres, B. A. (2018). Normal aging induces A1-like astrocyte reactivity. *Proc. Natl. Acad. Sci. U.S.A.* 115, E1896–E1905. doi: 10.1073/pnas.1800165115

Cohen, J., and Torres, C. (2019). Astrocyte senescence: evidence and significance. *Aging Cell* 18:e12937. doi: 10.1111/acel.12937

Conway, K. L., Kuballa, P., Khor, B., Zhang, M., Shi, H. N., Virgin, H. W., et al. (2013). ATG5 regulates plasma cell differentiation. *Autophagy* 9, 528–537. doi: 10.4161/auto.23484

Cornejo, F., Vruwink, M., Metz, C., Muñoz, P., Salgado, N., Poblete, J., et al. (2018). Scavenger receptor-A deficiency impairs immune response of microglia and astrocytes potentiating Alzheimer's disease pathophysiology. *Brain Behav. Immun.* 69, 336–350. doi: 10.1016/j.bbi.2017.12.007

Cotrina, M. L., and Nedergaard, M. (2002). Astrocytes in the aging brain. *J. Neurosci. Res.* 67, 1–10. doi: 10.1002/jnr.10121

Cressatti, M., Song, W., Turk, A. Z., Garabed, L. R., Benchaya, J. A., Galindez, C., et al. (2019). Glial HMOX1 expression promotes central and peripheral α-synuclein dysregulation and pathogenicity in parkinsonian mice. *Glia* 67, 1730–1744. doi: 10.1002/glia.23645

Cuervo, A. M. (2010). Chaperone-mediated autophagy: selectivity pays off. *Trends Endocrinol. Metab.* 21, 142–150. doi: 10.1016/j.tem.2009.10.003

De Groot, C. J. A., Bergers, E., Kamphorst, W., Ravid, R., Polman, C. H., and Barkhof, F. (2001). Post-mortem MRI-guided sampling of multiple sclerosis brain lesions: increased yield of active demyelinating and (p)reactive lesions. *Brain* 124, 1635–1645. doi: 10.1093/brain/124.8.1635

Deretic, V., Saitoh, T., and Akira, S. (2013). Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.* 13, 722–737. doi: 10.1038/nri3532

Djajadikerta, A., Keshri, S., Pavel, M., Prestil, R., Ryan, L., and Rubinsztein, D. C. (2020). Autophagy induction as a therapeutic strategy for neurodegenerative diseases. *J. Mol. Biol.* 432, 2799–2821. doi: 10.1016/j.jmb.2019.12.035

Dobson, R., and Giovannoni, G. (2019). Multiple Sclerosis–a review. *Eur. J. Neurol.* 26, 27–40. doi: 10.1111/ene.13819

Druart, M., and Le Magueresse, C. (2019). Emerging roles of complement in psychiatric disorders. *Front. Psychiatry* 10:573. doi: 10.3389/fpsyt.2019.00573

Duddy, M., Niino, M., Adatia, F., Hebert, S., Freedman, M., Atkins, H., et al. (2007). Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J. Immunol.* 178, 6092–6099. doi: 10.4049/jimmunol.178.10.6092

Dunham, J., van Driel, N., Eggen, B. J., Paul, C., 't Hart, B. A., Laman, J. D., et al. (2017). Analysis of the Cross-Talk of Epstein–Barr virus-infected B cells with T cells in the marmoset. *Clin. Transl. Immunol.* 6:e127. doi: 10.1038/cti.2017.1

Durafourt, B. A., Moore, C. S., Zammit, D. A., Johnson, T. A., Zaguia, F., Guiot, M. C., et al. (2012). Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia* 60, 717–727. doi: 10.1002/glia.22298

Dutta, R., and Trapp, B. D. (2014). Relapsing and progressive forms of multiple sclerosis: insights from pathology. *Curr. Opin. Neurol.* 27, 271–278. doi: 10.1097/WCO.0000000000000094

Dyment, D. A., Yee, I. M., Ebers, G. C., Sadovnick, A. D., and Canadian Collaborative Study Group (2006). Multiple sclerosis in stepsiblings: recurrence risk and ascertainment. *J. Neurol. Neurosurg. Psychiatry* 77, 258–259. doi: 10.1136/jnnp.2005.063008

Esposito, M., Ruffini, F., Bellone, M., Gagliani, N., Battaglia, M., Martino, G., et al. (2010). Rapamycin inhibits relapsing experimental autoimmune encephalomyelitis by both effector and regulatory T cells modulation. *J. Neuroimmunol.* 220, 52–63. doi: 10.1016/j.jneuroim.2010.01.001

Feng, X., Hou, H., Zou, Y., and Guo, L. (2017). Defective autophagy is associated with neuronal injury in a mouse model of multiple sclerosis. *Bosn. J. Basic Med. Sci.* 17, 95–103. doi: 10.17305/bjbms.2017.1696

Feng, X. D., Yu, S. S., Hou, H. Q., Zou, Y. L., Chen, J. J., and Guo, L. (2018). Rapamycin reduces degeneration of neurons by inhibiting Akt/MTOR/P70S6K pathway and restoring autophagy in EAE mice. *Int. J. Clin. Exp. Med.* 11, 3504–3513.

Feng, Y., He, D., Yao, Z., and Klionsky, D. J. (2014). The machinery of macroautophagy. *Cell Res.* 24, 24–41. doi: 10.1038/cr.2013.168

Ferguson, B., Matyszak, M. K., Esiri, M. M., and Perry, V. H. (1997). Axonal damage in acute multiple sclerosis lesions. *Brain* 120, 393–399. doi: 10.1093/brain/120.3.393

Fletcher, J. M., Lalor, S. J., Sweeney, C. M., Tubridy, N., and Mills, K. H. G. (2010). T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin. Exp. Immunol.* 162, 1–11. doi: 10.1111/j.1365-2249.2010.04143.x

François, A., Terro, F., Quellard, N., Fernandez, B., Chassaing, D., Janet, T., et al. (2014). Impairment of autophagy in the central nervous system during lipopolysaccharide-induced inflammatory stress in mice. *Mol. Brain* 7:56. doi: 10.1186/s13041-014-0056-z

Friese, M. A., and Fugger, L. (2009). Pathogenic CD8 + T cells in multiple sclerosis. *Ann. Neurol.* 66, 132–141. doi: 10.1002/ana.21744

Fujikake, N., Shin, M., and Shimizu, S. (2018). Association between autophagy and neurodegenerative diseases. *Front. Neurosci.* 12:255. doi: 10.3389/fnins.2018.00255

Gelfand, J. M., Cree, B. A. C., and Hauser, S. L. (2017). Ocrelizumab and other CD20+ B-cell-depleting therapies in multiple sclerosis. *Neurotherapeutics* 14, 835–841. doi: 10.1007/s13311-017-0557-4

Giunti, D., Parodi, B., Cordano, C., Uccelli, A., and Kerlero de Rosbo, N. (2014). Can we switch microglia's phenotype to foster neuroprotection? Focus on multiple sclerosis. *Immunology* 141, 328–339. doi: 10.1111/imm.12177

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., and Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell* 140, 918–934. doi: 10.1016/j.cell.2010.02.016

Gray, E., Thomas, T. L., Betmouni, S., Scolding, N., and Love, S. (2008). Elevated myeloperoxidase activity in white matter in multiple sclerosis. *Neurosci. Lett.* 444, 195–198. doi: 10.1016/j.neulet.2008.08.035

Greter, M., Heppner, F. L., Lemos, M. P., Odermatt, B. M., Goebels, N., Laufer, T., et al. (2005). Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat. Med.* 11, 328–334. doi: 10.1038/nm1197

Group Nature Publishing (2001). Multiple sclerosis: a two-stage Disease. *Nat. Immunol.* 2, 762–764. doi: 10.1038/ni0901-762

Guan, Y., Jakimovski, D., Ramanathan, M., Weinstock-Guttman, B., and Zivadinov, R. (2019). The Role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to in vivo imaging. *Neural Regen. Res.* 14, 373–386. doi: 10.4103/1673-5374.245462

Guerrero, B. L., and Sicotte, N. L. (2020). Microglia in multiple sclerosis: friend or foe? *Front. Immunol.* 11:374. doi: 10.3389/fimmu.2020.00374

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in Mice. *Nature* 441, 885–889. doi: 10.1038/nature04724

Harris, J., De Haro , S. A., Master, S. S., Keane, J., Roberts, E. A., Delgado, M., et al. (2007). T helper 2 cytokines inhibit autophagic control of intracellular mycobacterium tuberculosis. *Immunity* 27, 505–517. doi: 10.1016/j.immuni.2007.07.022

Harris, J., and Keane, J. (2010). How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clin. Exp. Immunol.* 161, 1–9. doi: 10.1111/j.1365-2249.2010.04146.x

Hassanpour, M., Hajihassani, F., Hiradfar, A., Aghamohammadzadeh, N., Rahbarghazi, R., Safaie, N., et al. (2020). Real-state of autophagy signaling pathway in neurodegenerative disease; focus on multiple sclerosis. *J. Inflamm.* 17, 1–8. doi: 10.1186/s12950-020-0237-8

Hauser, S. L., Waubant, E., Arnold, D. L., Vollmer, T., Antel, J., Fox, R. J., et al. (2008). B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N. Engl. J. Med.* 358, 676–688. doi: 10.1056/NEJMoa0706383

He, C., and Klionsky, D. J. (2009). Regulation mechanisms and signaling pathways of autophagy. *Ann. Rev. Genet.* 43, 67–93. doi: 10.1146/annurev-genet-102808-114910

He, Y., She, H., Zhang, T., Xu, H., Cheng, L., Yepes, M., et al. (2018). P38 MAPK inhibits autophagy and promotes microglial inflammatory responses by phosphorylating ULK1. *J. Cell Biol.* 217, 315–328. doi: 10.1083/jcb.201701049

Heppner, F. L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hövelmeyer, N., et al. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat. Med.* 11, 146–152. doi: 10.1038/nm1177

Hill, R. A., Li, A. M., and Grutzendler, J. (2018). Lifelong cortical myelin plasticity and age-related degeneration in the live mammalian brain. *Nat. Neurosci.* 21, 683–695. doi: 10.1038/s41593-018-0120-6

Hirotani, M., Niino, M., Fukazawa, T., Kikuchi, S., Yabe, I., Hamada, S., et al. (2010). Decreased IL-10 production mediated by toll-like receptor 9 in B cells in multiple sclerosis. *J. Neuroimmunol.* 221, 95–100. doi: 10.1016/j.jneuroim.2010.02.012

Hubbard, V. M., Valdor, R., Patel, B., Singh, R., Cuervo, A. M., and Macian, F. (2010). Macroautophagy regulates energy metabolism during effector T cell activation. *J. Immunol.* 185, 7349–7357. doi: 10.4049/jimmunol.1000576

Igci, M., Baysan, M., Yigiter, R., Ulasli, M., Geyik, S., Bayraktar, R., et al. (2016). Gene expression profiles of autophagy-related genes in multiple sclerosis. *Gene* 588, 38–46. doi: 10.1016/j.gene.2016.04.042

Ireland, J. M., and Unanue, E. R. (2011). Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J. Exp. Med.* 208, 2625–2632. doi: 10.1084/jem.20110640

Jagessar, S. A., Holtman, I. R., Hofman, S., Morandi, E., Heijmans, N., Laman, J. D., et al. (2016). Lymphocryptovirus infection of nonhuman primate B cells converts destructive into productive processing of the pathogenic CD8 T cell epitope in myelin oligodendrocyte glycoprotein. *J. Immunol.* 197, 1074–1088. doi: 10.4049/jimmunol.1600124

Jakimovski, D., Weinstock-Guttman, B., Ramanathan, M., Kolb, C., Hojnacki, D., Minagar, A., et al. (2017). Ocrelizumab: a B-cell depleting therapy for multiple sclerosis. *Expert Opin. Biol. Ther.* 17, 1163–1172. doi: 10.1080/14712598.2017.1347632

Jang, S. Y., Shin, Y. K., Park, S. Y., Park, J. Y., Lee, H. J., Yoo, Y. H., et al. (2016). Autophagic myelin destruction by schwann cells during wallerian degeneration and segmental demyelination. *Glia* 64, 730–742. doi: 10.1002/glia.22957

Jia, W., and He, Y. W. (2011). Temporal regulation of intracellular organelle homeostasis in T lymphocytes by autophagy. *J. Immunol.* 186, 5313–5322. doi: 10.4049/jimmunol.1002404

Jin, M. M., Wang, F., Qi, D., Liu, W. W., Gu, C., Mao, C. J., et al. (2018). A critical role of autophagy in regulating microglia polarization in neurodegeneration. *Front. Aging Neurosci.* 10:378. doi: 10.3389/fnagi.2018.00378

Joe, E. H., Choi, D. J., An, J., Eun, J. H., Jou, I., and Park, S. (2018). Astrocytes, microglia, and Parkinson's disease. *Exp. Neurobiol.* 27, 77–87. doi: 10.5607/en.2018.27.2.77

Keller, C. W., and Lünemann, J. D. (2017). Autophagy and autophagy-related proteins in CNS autoimmunity. *Front. Immunol.* 8:165. doi: 10.3389/fimmu.2017.00165

Keller, C. W., Sina, C., Kotur, M. B., Ramelli, G., Mundt, S., Quast, I., et al. (2017). ATG-dependent phagocytosis in dendritic cells drives myelin-specific CD4+ T cell pathogenicity during CNS inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 114, E11228–E11237. doi: 10.1073/pnas.1713664114

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1-and Irf8-dependent pathways. *Nat. Neurosci.* 16, 273–280. doi: 10.1038/nn.3318

Kim, H. J., Cho, M. H., Shim, W. H., Kim, J. K., Jeon, E. Y., Kim, D. H., et al. (2017). Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. *Mol. Psychiatry* 22, 1576–1584. doi: 10.1038/mp.2016.103

Kirkin, V., and Rogov, V. V. (2019). A diversity of selective autophagy receptors determines the specificity of the autophagy pathway. *Mol. Cell* 76, 268–285. doi: 10.1016/j.molcel.2019.09.005

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441, 880–884. doi: 10.1038/nature04723

Komatsu, M., Waguri, S., Koike, M., Sou, Y. S., Ueno, T., Hara, T., et al. (2007). Homeostatic levels of P62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* 131, 1149–1163. doi: 10.1016/j.cell.2007.10.035

Kovacs, J. R., Li, C., Yang, Q., Li, G., Garcia, I. G., Ju, S., et al. (2012). Autophagy promotes T-Cell survival through degradation of proteins of the cell death machinery. *Cell Death Differ.* 19, 144–152. doi: 10.1038/cdd.2011.78

Lahiri, V., Hawkins, W. D., and Klionsky, D. J. (2019). Watch what you (self-) eat: autophagic mechanisms that modulate metabolism. *Cell Metab.* 29, 803–826. doi: 10.1016/j.cmet.2019.03.003

Lai, S. C., and Devenish, R. J. (2012). LC3-associated phagocytosis (LAP): connections with host autophagy. *Cells* 1, 396–408. doi: 10.3390/cells1030396

Lampron, A., Larochelle, A., Laflamme, N., Préfontaine, P., Plante, M. M., Sánchez, M. G., et al. (2015). Inefficient clearance of myelin debris by microglia impairs remyelinating processes. *J. Exp. Med.* 212, 481–495. doi: 10.1084/jem.20141656

Lassmann, H., Brück, W., and Lucchinetti, C. F. (2007). The Immunopathology of Multiple Sclerosis: an Overview. *Brain Pathol.* 17, 210–218. doi: 10.1111/j.1750-3639.2007.00064.x

Law, A. H., Lee, D. C., Yuen, K. Y., Peiris, M., and Lau, A. S. (2010). Cellular response to influenza virus infection: a potential role for autophagy in CXCL10 and interferon-alpha induction. *Cell. Mol. Immunol.* 7, 263–270. doi: 10.1038/cmi.2010.25

Lee, H. Y., Mattei, L. M., Steinberg, B. E., Alberts, P., Lee, Y. H., Chervonsky, A., et al. (2010). In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* 32, 227–239. doi: 10.1016/j.immuni.2009.12.006

Lee, S. J., Cho, and Koh, J. Y. (2009). Oxidative injury triggers autophagy in astrocytes: the role of endogenous zinc. *Glia* 57, 1351–1361. doi: 10.1002/glia.20854

Lehmann Horn, K., Kronsbein, H. C., and Weber, M. S. (2013). Targeting B cells in the treatment of multiple sclerosis: recent advances and remaining challenges. *Therapeut. Adv. Neurol. Disord.* 6, 161–173. doi: 10.1177/1756285612474333

Levine, B., and Kroemer, G. (2019). Biological functions of autophagy genes: a disease perspective. *Cell* 176, 11–42. doi: 10.1016/j.cell.2018.09.048

Levine, B., Mizushima, N., and Virgin, H. W. (2011). Autophagy in immunity and inflammation. *Nature* 469, 323–335. doi: 10.1038/nature09782

Li, C., Capan, E., Zhao, Y., Zhao, J., Stolz, D., Watkins, S. C., et al. (2006). Autophagy is induced in CD4 + T Cells and important for the growth factor-withdrawal cell death. *J. Immunol.* 177, 5163–5168. doi: 10.4049/jimmunol.177.8.5163

Li, R., Patterson, K. R., and Bar-Or, A. (2018). Reassessing B cell contributions in multiple sclerosis. *Nat. Immunol.* 19, 696–707. doi: 10.1038/s41590-018-0135-x

Li, W. W., Li, J., and Bao, J. K. (2012). Microautophagy: lesser-known self-eating. *Cell. Mol. Life Sci.* 69, 1125–1136. doi: 10.1007/s00018-011-0865-5

Li, W. W., Setzu, A., Zhao, C., and Franklin, R. J. (2005). Minocycline-mediated inhibition of microglia activation impairs oligodendrocyte progenitor cell responses and remyelination in a non-immune model of demyelination. *J. Neuroimmunol.* 158, 58–66. doi: 10.1016/j.jneuroim.2004.08.011

Liang, P., and Le, W. (2015). Role of autophagy in the pathogenesis of multiple sclerosis. *Neurosci. Bull.* 31, 435–444. doi: 10.1007/s12264-015-1545-5

Lieberman, O. J., McGuirt, A. F., Tang, G., and Sulzer, D. (2019). Roles for neuronal and glial autophagy in synaptic pruning during development. *Neurobiol. Dis.* 122, 49–63. doi: 10.1016/j.nbd.2018.04.017

Lin, N. Y., Beyer, C., Gießl, A., Kireva, T., Scholtysek, C., Uderhardt, S., et al. (2013). Autophagy regulates TNFα-mediated joint destruction in experimental arthritis. *Ann. Rheum. Dis.* 72, 761–768. doi: 10.1136/annrheumdis-2012-201671

Liu, X., Tian, F., Wang, S., Wang, F., and Xiong, L. (2018). Astrocyte autophagy flux protects neurons against oxygen-glucose deprivation and ischemic/reperfusion injury. *Rejuvenat. Res.* 21, 405–415. doi: 10.1089/rej.2017.1999

Livingston, P. G., Kurane, I., and Ennis, F. A. (1997). Use of epstein-barr virus-transformed, autologous B-Lymphoblastoid cells as antigen-presenting cells for establishment and maintenance of dengue virus-specific, human cytotoxic T lymphocyte clones. *J. Virol. Methods* 67, 77–84. doi: 10.1016/S0166-0934(97)00082-7

Lloyd, A. F., Davies, C. L., and Miron, V. E. (2017). Microglia: origins, homeostasis, and roles in myelin repair. *Curr. Opin. Neurobiol.* 47, 113–120. doi: 10.1016/j.conb.2017.10.001

Lovas, G., Szilágyi, N., Majtényi, K., Palkovits, M., and Komoly, S. (2000). Axonal changes in chronic demyelinated cervical spinal cord plaques. *Brain* 123, 308–317. doi: 10.1093/brain/123.2.308

Luo, C., Jian, C., Liao, Y., Huang, Q., Wu, Y., Liu, X., et al. (2017). The role of microglia in multiple sclerosis. *Neuropsych. Dis. Treat.* 13, 1661–1667. doi: 10.2147/NDT.S140634

Macian, F. (2019). Autophagy in T cell function and aging. *Front. Cell Dev. Biol.* 7:2013. doi: 10.3389/fcell.2019.00213

Malta, C. D., Fryer, J. D., Settembre, C., and Ballabio, A. (2012). Astrocyte dysfunction triggers neurodegeneration in a lysosomal storage disorder. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2334–E2342. doi: 10.1073/pnas.1209577109

Martinez-Vicente, M., and Cuervo, A. M. (2007). Autophagy and neurodegeneration: when the cleaning crew goes on strike. *Lancet Neurol.* 6, 352–361. doi: 10.1016/S1474-4422(07)70076-5

Meikle, L., Pollizzi, K., Egnor, A., Kramvis, I., Lane, H., Sahin, M., et al. (2008). Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (MTOR) inhibitors: effects on MTORC1 and Akt signaling lead to improved survival and function. *J. Neurosci.* 28, 5422–5432. doi: 10.1523/JNEUROSCI.0955-08.2008

Melief, J., Koning, N., Schuurman, K. G., Van De Garde, M. D., Smolders, J., Hoek, R. M., et al. (2012). Phenotyping Primary human microglia: tight regulation of LPS responsiveness. *Glia* 60, 1506–1517. doi: 10.1002/glia.22370

Melief, J., Schuurman, K. G., Van de Garde, M. D., Smolders, J., Van Eijk, M., Hamann, J., et al. (2013). Microglia in normal appearing white matter of multiple sclerosis are alerted but immunosuppressed. *Glia* 61, 1848–1861. doi: 10.1002/glia.22562

Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., et al. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. *Neuron* 93, 1015–1034. doi: 10.1016/j.neuron.2017.01.022

Miller, B. C., Zhao, Z., Stephenson, L. M., Cadwell, K., Pua, H. H., Lee, H. K., et al. (2008). The autophagy gene ATG5 plays an essential role in B lymphocyte development. *Autophagy* 4, 309–314. doi: 10.4161/auto.5474

Miron, V. E., Boyd, A., Zhao, J. W., Yuen, T. J., Ruckh, J. M., Shadrach, J. L., et al. (2013). M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat. Neurosci.* 16, 1211–1218. doi: 10.1038/nn.3469

Mizushima, N., Levine, B., Cuervo, A. M., and Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature* 451, 1069–1075. doi: 10.1038/nature06639

Mohammad, M. G., Hassanpour, M., Tsai, V. W., Li, H., Ruitenberg, M. J., Booth, D. W., et al. (2013). Dendritic Cells and multiple sclerosis: disease, tolerance and therapy. *Int. J. Mol. Sci.* 14, 547–562. doi: 10.3390/ijms14010547

Morandi, E., Jagessar, S. A., t Hart, B. A., and Bruno, B. (2017). EBV infection empowers human B cells for autoimmunity: role of autophagy and relevance to multiple sclerosis. *J. Immunol.* 199, 435–448. doi: 10.4049/jimmunol.1700178

Morselli, E., Mariño, G., Bennetzen, M. V., Eisenberg, T., Megalou, E., Schroeder, S., et al. (2011). Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. *J. Cell Biol.* 192, 615–629. doi: 10.1083/jcb.201008167

Motori, E., Puyal, J., Toni, N., Ghanem, A., Angeloni, C., Malaguti, M., et al. (2013). Inflammation-induced alteration of astrocyte mitochondrial dynamics requires autophagy for mitochondrial network maintenance. *Cell Metab.* 18, 844–859. doi: 10.1016/j.cmet.2013.11.005

Mulero, P., Midaglia, L., and Montalban, X. (2018). Ocrelizumab: a new milestone in multiple sclerosis therapy. *Therap. Adv. Neurol. Disord.* 11, 1–6. doi: 10.1177/1756286418773025

Muller, S., Brun, S., René, F., de Sèze, J., Loeffler, J. P., and Jeltsch-David, H. (2017). Autophagy in neuroinflammatory diseases. *Autoimmun. Rev.* 16, 856–874. doi: 10.1016/j.autrev.2017.05.015

Münz, C. (2016). Autophagy proteins in antigen processing for presentation on MHC molecules. *Immunol. Rev.* 272, 17–27. doi: 10.1111/imr.12422

Nakatogawa, H. (2020). Mechanisms governing autophagosome biogenesis. *Nat. Rev. Mol. Cell Biol.* 21, 439–458. doi: 10.1038/s41580-020-0241-0

Nave, K. A., and Werner, H. B. (2014). Myelination of the Nervous system: mechanisms and functions. *Annu. Rev. Cell Dev. Biol.* 30, 503–533. doi: 10.1146/annurev-cellbio-100913-013101

Nissen, J. C., Thompson, K. K., West, B. L., and Tsirka, S. E. (2018). Csf1R inhibition attenuates experimental autoimmune encephalomyelitis and

promotes recovery. *Exp. Neurol.* 307, 24–36. doi: 10.1016/j.expneurol.2018.05.021

Nixon, R. A. (2013). The role of autophagy in neurodegenerative disease. *Nat. Med.* 19, 983–997. doi: 10.1038/nm.3232

Nixon, R. A., and Yang, D. S. (2011). Autophagy failure in Alzheimer's disease-locating the primary defect. *Neurobiol. Dis.* 43, 38–45. doi: 10.1016/j.nbd.2011.01.021

Nuyts, A. H., Lee, W. P., Bashir-Dar, R., Berneman, Z. N., and Cools, N. (2013). Dendritic cells in multiple sclerosis: key players in the Immunopathogenesis, key players for new cellular immunotherapies? *Mult. Scler. J.* 19, 995–1002. doi: 10.1177/1352458512473189

Orihuela, R., McPherson, C. A., and Harry, G. J. (2016). Microglial M1/M2 polarization and metabolic states. *Br. J. Pharmacol.* 173, 649–665. doi: 10.1111/bph.13139

Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T., et al. (2005). Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 307, 593–596. doi: 10.1126/science.1104904

Parekh, V. V., Wu, L., Kelli, L., Janice, A. B., Jennifer, A. W., Olivares-Villagómez, G. D., et al. (2013). Impaired autophagy, defective T cell homeostasis, and a wasting syndrome in mice with a T cell–specific deletion of Vps34. *J. Immunol.* 190, 5086–5101. doi: 10.4049/jimmunol.1202071

Park, H. J., Lee, S. J., Kim, S. H., Han, J., Bae, J., Kim, S. J., et al. (2011). IL-10 Inhibits the starvation induced autophagy in macrophages via class i phosphatidylinositol 3-kinase (PI3K) pathway. *Mol. Immunol.* 48, 720–727. doi: 10.1016/j.molimm.2010.10.020

Patsopoulos, N. A., Baranzini, S. E., Santaniello, A., Shoostari, P., Cotsapas, C., Wong, G., et al. (2019). Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science* 365:6460. doi: 10.1126/science.aav7188

Paunovic, V., Petrovic, I. V., Milenkovic, M., Janjetovic, K., Pravica, V., Dujmovic, I., et al. (2018). Autophagy-independent increase of ATG5 expression in T cells of multiple sclerosis patients. *J. Neuroimmunol.* 319 100–105. doi: 10.1016/j.jneuroim.2018.03.001

Pengo, N., Scolari, M., Oliva, L., Milan, E., Mainoldi, F., Raimondi, A., et al. (2013). Plasma cells require autophagy for sustainable immunoglobulin production. *Nat. Immunol.* 14, 298–305. doi: 10.1038/ni.2524

Plaza-Zabala, A., Sierra-Torre, V., and Sierra, A. (2017). Autophagy and microglia: novel partners in neurodegeneration and aging. *Int. J. Mol. Sci.* 18:598. doi: 10.3390/ijms18030598

Ponath, G., Park, C., and Pitt, D. (2018). The Role of astrocytes in multiple sclerosis. *Front. Immunol.* 9:217. doi: 10.3389/fimmu.2018.00217

Ponomarev, E. D., Leah, P. S., Maresz, K., and Bonnie, N. D. (2005). Microglial cell activation and proliferation precedes the onset of CNS autoimmunity. *J. Neurosci. Res.* 81, 374–389. doi: 10.1002/jnr.20488

Prineas, J. W., Kwon, E. E., Sook, E., Cho, L. R., Sharer, M. H., Barnett, E. L., et al. (2001). Immunopathology of secondary-progressive multiple sclerosis. *Ann. Neurol.* 50, 646–657. doi: 10.1002/ana.1255

Pua, H. H., Dzhagalov, I., Chuck, M., Mizushima, N., and He, Y. W. (2007). A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J. Exp. Med.* 204, 25–31. doi: 10.1084/jem.20061303

Pua, H. H., Guo, J., Komatsu, M., and He, Y.-W. (2009). Autophagy Is essential for mitochondrial clearance in mature T lymphocytes. *J. Immunol.* 182, 4046–4055. doi: 10.4049/jimmunol.0801143

Pua, H. H., and He, Y. W. (2007). Maintaining T lymphocyte homeostasis: another duty of autophagy. *Autophagy* 3, 266–267. doi: 10.4161/auto.3908

Puente, C., Hendrickson, R. C., and Jiang, X. (2016). Nutrient-regulated phosphorylation of ATG13 inhibits starvation-induced autophagy. *J. Biol. Chem.* 291, 6026–6035. doi: 10.1074/jbc.M115.689646

Puleston, D. J., and Simon, A. K. (2014). Autophagy in the immune system. *Immunology* 141, 1–8. doi: 10.1111/imm.12165

Qian, M., Fang, X., and Wang, X. (2017). Autophagy and inflammation. *Clin. Trans. Med.* 6:24. doi: 10.1186/s40169-017-0154-5

Ramaglia, V., Hughes, T. R., Donev, R. M., Ruseva, M. M., Wu, X., Huitinga, I., et al. (2012). C3-dependent mechanism of microglial priming relevant to multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 109, 965–970. doi: 10.1073/pnas.1111924109

Rangaraju, S., Verrier, J. D., Madorsky, I., Nicks, J., Dunn, W. A., and Notterpek, L. (2010). Rapamycin activates autophagy and improves myelination in explant cultures from neuropathic mice. *J. Neurosci.* 30, 11388–11397. doi: 10.1523/JNEUROSCI.1356-10.2010

Rathmell, J. C. (2012). Metabolism and autophagy in the immune system: immunometabolism comes of age. *Immunol. Rev.* 249, 5–13. doi: 10.1111/j.1600-065X.2012.01158.x

Ravikumar, B., Moreau, K., Jahreiss, L., Puri, C., and Rubinsztein, D. C. (2010). Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat. Cell Biol.* 12, 747–757. doi: 10.1038/ncb2078

Ravikumar, B., Vacher, C., Berger, Z., Davies, J. E., Luo, S., Oroz, L. G., et al. (2004). Inhibition of MTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of huntington disease. *Nat. Genet.* 36, 585–595. doi: 10.1038/ng1362

Rezai-Zadeh, K., Gate, D., and Town, T. (2009). CNS infiltration of peripheral immune cells: D-day for neurodegenerative disease? *J. Neuroim. Pharmacol.* 4, 462–475. doi: 10.1007/s11481-009-9166-2

Rubinsztein, D. C., Bento, C. F., and Deretic, V. (2015). Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. *J. Exp. Med.* 212, 979–990. doi: 10.1084/jem.20150956

Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B. G., Satoh, T., et al. (2008). Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. *Nature* 456, 264–268. doi: 10.1038/nature07383

Salminen, A., Kaarniranta, K., and Kauppinen, A. (2013). Beclin 1 interactome controls the crosstalk between apoptosis, autophagy and inflammasome activation: impact on the aging process. *Ageing Res. Rev.* 12, 520–534. doi: 10.1016/j.arr.2012.11.004

Salou, M., Nicol, B., Garcia, A., and Laplaud, D. A. (2015). Involvement of CD8+ T cells in multiple sclerosis. *Front. Immunol.* 6:604. doi: 10.3389/fimmu.2015.00604

Sanjuan, M. A., Dillon, C. P., Stephen, W. G., Moshiach, T. S., Dorsey, F., Connell, S., et al. (2007). Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 450, 1253–1257. doi: 10.1038/nature06421

Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C. A., Patsopoulos, N. A., Moutsianas, L., et al. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219. doi: 10.1038/nature10251

Schmid, D., Park, C. G., Hartl, C. A., Subedi, N., Cartwright, A. N., Puerto, R. B., et al. (2017). T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. *Nat. Commun.* 8, 1–11. doi: 10.1038/s41467-017-01830-8

Schmid, D., Pypaert, M., and Münz, C. (2007). Antigen-loading compartments for major histocompatibility complex class II Molecules continuously receive input from autophagosomes. *Immunity* 26, 79–92. doi: 10.1016/j.immuni.2006.10.018

Schulz, C., Perdiguero, E. G., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 335, 86–90. doi: 10.1126/science.1219179

Shao, B. Z., Wei, W., Ke, P., Xu, Z. Q., Zhou, J. X., and Liu, C. (2014). Activating cannabinoid receptor 2 alleviates pathogenesis of experimental autoimmune encephalomyelitis via activation of autophagy and inhibiting NLRP3 inflammasome. *CNS Neurosci. Therapeut.* 20, 1021–1028. doi: 10.1111/cns.12349

Shi, C. S., Shenderov, K., Huang, N. N., Kabat, J., Abu-Asab, M., Fitzgerald, K. A., et al. (2012). Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. *Nat. Immunol.* 13, 255–263. doi: 10.1038/ni.2215

Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G. R., Zou, Z., Kinch, L., et al. (2013). Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* 494, 201–206. doi: 10.1038/nature11866

Skripuletz, T., Hackstette, D., Bauer, K., Gudi, V., Pul, R., Voss, E., et al. (2013). Astrocytes regulate myelin clearance through recruitment of microglia during cuprizone-induced demyelination. *Brain* 136, 147–167. doi: 10.1093/brain/aws262

Smith, C. M., Mayer, J. A., and Duncan, I. D. (2013). Autophagy promotes oligodendrocyte survival and function following dysmyelination in a long-lived myelin mutant. *J. Neurosci.* 33, 8088–8100. doi: 10.1523/JNEUROSCI.0233-13.2013

Sofroniew, M. V., and Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol.* 119, 7–35. doi: 10.1007/s00401-009-0619-8

Sospedra, M. (2018). B cells in multiple sclerosis. *Curr. Opin. Neurol.* 31, 256–262. doi: 10.1097/WCO.0000000000000563

Stamatakou, E., Wróbel, L., Hill, S. M., Puri, C., Son, S. M., Fujimaki, M., et al. (2020). Mendelian neurodegenerative disease genes involved in autophagy. *Cell Discov.* 6:158. doi: 10.1038/s41421-020-0158-y

Stavoe, A. K. H., and Holzbaur, E. L. F. (2019). Autophagy in neurons. *Ann. Rev. Cell Dev. Biol.* 35, 477–500. doi: 10.1146/annurev-cellbio-100818-125242

Stoffels, J. M. J., De Jonge, J. C., Stancic, M., Nomden, A., Van Strien, M. E., Šišková, D. M. Z., et al. (2013). Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. *Brain* 136, 116–131. doi: 10.1093/brain/aws313

Su, P., Zhang, J., Wang, D., Zhao, F., Cao, Z., Aschner, M., et al. (2016). The role of autophagy in modulation of neuroinflammation in microglia. *Neuroscience* 319, 155–167. doi: 10.1016/j.neuroscience.2016.01.035

't Hart, B. A., Kap, Y. S., Morandi, E., Laman, J. D., and Gran, B. (2016). EBV infection and multiple sclerosis: lessons from a marmoset model. *Trends Mol. Med.* 22, 1012–1024. doi: 10.1016/j.molmed.2016.10.007

Thorley-Lawson, D. A., and Mann, K. P. (1985). Early events in epstein-barr virus infection provide a model for b cell activation. *J. Exp. Med.* 162, 45–59. doi: 10.1084/jem.162.1.45

Trapp, B. D., and Nave, K. A. (2008). Multiple sclerosis: an immune or neurodegenerative disorder? *Ann. Rev. Neurosci.* 31, 247–269. doi: 10.1146/annurev.neuro.30.051606.094313

Trapp, B. D., Peterson, J., Ransohoff, R. M., Rudick, R., Mörk, S., and Bö, L. (1998). Axonal transection in the lesions of multiple sclerosis. *New Engl. J. Med.* 338, 278–285. doi: 10.1056/NEJM199801293380502

Tsunoda, I., and Fujinami, R. S. (2002). Inside-out versus outside-in models for virus induced demyelination: axonal damage triggering demyelination. *Springer Semin. Immunopathol.* 24, 105–125. doi: 10.1007/s00281-002-0105-z

Tsunoda, I., Kuang, L. Q., Libbey, J. E., and Fujinami, R. S. (2003). Axonal injury heralds virus-induced demyelination. *Am. J. Pathol.* 162, 1259–1269. doi: 10.1016/S0002-9440(10)63922-3

van Beek, N., Klionsky, D., and Reggiori, F. (2018). Genetic aberrations in macroautophagy genes leading to diseases. *Biochim. Biophys. Acta Mol. Cell Res.* 1865, 803–816. doi: 10.1016/j.bbamcr.2018.03.002

Varshney, P., and Saini, N. (2018). PI3K/AKT/MTOR activation and autophagy inhibition plays a key role in increased cholesterol during IL-17A mediated inflammatory response in psoriasis. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 1795–1803. doi: 10.1016/j.bbadis.2018.02.003

von Büdingen, H. C., Palanichamy, A., Lehmann-Horn, K., Michel, B. A., and Zamvil, S. S. (2015). Update on the autoimmune pathology of multiple sclerosis: B-cells as disease-drivers and therapeutic targets. *Eur. Neurol.* 73, 238–246. doi: 10.1159/000377675

Voß, E. V., Škuljec, J., Gudi, V., Skripuletz, T., Pul, R., Trebst, C., et al. (2012). Characterisation of microglia during De- and remyelination: can they create a repair promoting environment? *Neurobiol. Dis.* 45, 519–528. doi: 10.1016/j.nbd.2011.09.008

Wang, J., Wang, J., Wang, J., Yang, B., Weng, Q., and He, Q. (2019). Targeting microglia and macrophages: a potential treatment strategy for multiple sclerosis. *Front. Pharmacol.* 10:286. doi: 10.3389/fphar.2019.00286

Wang, J. L., and Xu, C. J. (2020). Astrocytes autophagy in aging and neurodegenerative disorders. *Biomed. Pharmacother.* 122:109691. doi: 10.1016/j.biopha.2019.109691

Wang, S., Li, B., Qiao, H., Lv, X., Liang, Q., Shi, Z., et al. (2014). Autophagy−related Gene Atg5 is essential for astrocyte differentiation in the developing mouse cortex. *EMBO Rep.* 15, 1053–1061. doi: 10.15252/embr.201338343

Werneburg, S., Jung, J., Kunjamma, R. B., Ha, S. K., Luciano, N. J., Willis, C. M., et al. (2020). Targeted complement inhibition at synapses prevents microglial synaptic engulfment and synapse loss in demyelinating disease. *Immunity* 52, 167–182.e7. doi: 10.1016/j.immuni.2019.12.004

Wolswijk, G. (2000). Oligodendrocyte Survival, loss and birth in lesions of chronic-stage multiple sclerosis. *Brain* 123, 105–115. doi: 10.1093/brain/123.1.105

Wolswijk, G. (2002). Oligodendrocyte precursor cells in the demyelinated multiple sclerosis spinal cord. *Brain* 125, 338–349. doi: 10.1093/brain/awf031

Wong, E., and Cuervo, A. M. (2010). Autophagy gone awry in neurodegenerative diseases. *Nat. Neurosci.* 13, 805–811. doi: 10.1038/nn.2575

Wong, Y. C., and Holzbaur, E. L. F. (2014). Optineurin Is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proc. Natl. Acad. Sci. U.S.A.* 111, E4439–E4448. doi: 10.1073/pnas.1405752111

Yang, Z., Goronzy, J. J., and Weyand, C. M. (2015). Autophagy in autoimmune disease. *J. Mol. Med.* 93, 707–717. doi: 10.1007/s00109-015-1297-8

Yin, H., Wu, H., Chen, Y., Zhang, J., Zheng, M., Chen, G., et al. (2018). The therapeutic and pathogenic role of autophagy in autoimmune diseases. *Front. Immunol.* 9:1512. doi: 10.3389/fimmu.2018.01512

Yogev, N., Frommer, F., Lukas, D., Kautz-Neu, K., Karram, K., Ielo, D., et al. (2012). Dendritic Cells ameliorate autoimmunity in the cns by controlling the homeostasis of PD-1 receptor+ regulatory T cells. *Immunity* 37, 264–275. doi: 10.1016/j.immuni.2012.05.025

Zachari, M., and Ganley, I. G. (2017). The mammalian ULK1 complex and autophagy initiation. *Essays Biochem.* 61, 585–596. doi: 10.1042/EBC20170021

Zeis, T., Probst, A., Steck, A. J., Stadelmann, C., Brück, W., and Schaeren-Wiemers, N. (2009). Molecular changes in white matter adjacent to an active demyelinating lesion in early multiple sclerosis: molecular changes in MS periplaque white matter. *Brain Pathol.* 19, 459–466. doi: 10.1111/j.1750-3639.2008.00231.x

Zhang, F., Lin, Y. A., Kannan, S., and Kannan, R. M. (2016). Targeting specific cells in the brain with nanomedicines for CNS therapies. *J. Control. Release* 240, 212–226. doi: 10.1016/j.jconrel.2015.12.013

Zhang, H., Puleston, D. J., and Simon, A. K. (2016). Autophagy and immune senescence. *Trends Mol. Med.* 22, 671–686. doi: 10.1016/j.molmed.2016.06.001

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OPINION
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Cysteine Proteases and Mitochondrial Instability: A Possible Vicious Cycle in MS Myelin?

*Anthony Poerwoatmodjo, Geert J. Schenk, Jeroen J. G. Geurts and Antonio Luchicchi**

Division Clinical Neurosciences, Department of Anatomy and Neurosciences, Amsterdam Neuroscience, Amsterdam Universitair Medische Centra (UMC), Location Vrije Universiteit (VU) Medical Center, MS Center Amsterdam, Amsterdam, Netherlands

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****Correspondence:***
Antonio Luchicchi
a.luchicchi@amsterdamumc.nl

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INTRODUCTION

Multiple sclerosis (MS) is a chronic neuroinflammatory disease of the central nervous system (CNS) of which the main pathological feature is the loss of white matter myelin (demyelination) (Ringold et al., 2005).

Although a solid cause to explain MS origin is still debated, a classic etiological view (named *outside-in* hypothesis of MS) regards this disorder as primarily mediated by a faulty $CD4^+$ T-lymphocytic attack against the myelin (Lassmann and Ransohoff, 2004; Chitnis, 2007).

Despite its prevalence, this hypothesis has been recently questioned by an opposing view (*inside-out* hypothesis of MS) which posits that subtle primary cytodegenerative processes might happen in the CNS itself, leading to myelin disintegration and a secondary immune system response (Stys et al., 2012). For instance, studies have shown that MS pathological correlates, like widening of myelin lamellae and oligodendrocyte apoptosis, are often present in regions separated form inflammation foci (Barnett and Prineas, 2004; Henderson et al., 2009), and that loss of inner myelin sheath-expressed proteins (like the myelin associated glycoprotein, MAG) temporarily precedes that of outer-sheath expressed molecules in newly forming lesions (Aboul-Enein et al., 2003). Furthermore, clinical observations have recurrently reported that immunosuppressant agents, elective to treat relapsing-remitting forms of MS, are largely ineffective in halting less inflammatory progressive forms of the disease (Stys et al., 2012).

Although a solid sequence of events is still elusive, one putative condition to explain this primary myelin degeneration in MS may be the subtle imbalance at the level of the axon-myelinic synapse (AMS, **Figure 1A**; Micu et al., 2018). The AMS is a recently proposed form of glutamate-mediated communication between axon and myelin which regulates axonal myelination and action potential propagation via n-methyl-d-aspartate (NMDA) receptor-dependent myelin Ca^{2+} supply (**Figure 1A**, 1–3; Micu et al., 2018). Briefly, controlled myelinic Ca^{2+} influx takes part in glycolysis processes which lead to pyruvate and lactate production (**Figure 1A**, 4). Lactate is then back-transported to the axon where it boosts energy production during electric activity (**Figure 1A**, 5).

Consequently, alterations at the level of the AMS, triggered by aberrant stimuli like dysfunctional Ca^{2+} mobilization from the axoplasmic reticulum (Micu et al., 2018), Na^+ homeostasis alterations (Inglese et al., 2010) and/or the pathological presence of axonal

nanoruptures (Witte et al., 2019) (**Figure 1B**, a–c) may instigate an augmented myelinic Ca^{2+} entrance. As elegantly shown in studies employing cuprizone mouse models of MS, this condition is responsible of many aberrant biochemical processes relevant for myelin stability including the citrullination of the myelin basic protein (MBP) (Caprariello et al., 2018). Citrullination is a posttranslational modification process instigated by the activation of the enzyme protein arginine deiminase (PAD) that promotes the formation of highly immunogenic myelin (**Figure 1B**, 1–3).

Several parallel aberrant processes may lead citrullinated myelin to degrade. Among them, the activation of cysteine proteases like the Ca^{2+}-dependent non-lysosomal protease calpain and the lysosomal Ca^{2+}-independent cathepsin might very well-recapitulate MS myelin degenerative processes.

Calpain and cathepsin play a role in apoptotic processes (Leist and Jaattela, 2001; Kroemer and Jaattela, 2005), Na^{+}/Ca^{2+} exchange pump/actin cytoskeleton degradation (McConkey, 1998; Zong and Thompson, 2006), and myelin retraction from the node of Ranvier (Baraban et al., 2018). Moreover, studies on ischemia and sporadic Creutzfeldt-Jakob disease have shown that, under specific circumstances (e.g., aberrant cellular Ca^{2+} influx), these two proteases engage into a sequential activation named "*calpain-cathepsin axis*" where calpain promotes a cleavage of lysosomal associated membrane 2 (LAMP2). As a result, a programmed cell death through lysosomal cathepsin leakage is activated (Miyazawa et al., 1998; Zong and Thompson, 2006; Villalpando Rodriguez and Torriglia, 2013; Llorens et al., 2017).

This axial activation, combined with the hypothesized role of cathepsin in degrading the MAG (Stebbins et al., 1997), an inner-sheath adhesion molecule important for axon-myelinic stability (Trapp and Quarles, 1982; Pronker et al., 2016), may explain the primary degenerative processes involved in MS-related AMS instability and myelin disintegration.

Despite this, whether an aberrant cysteine protease activation is relevant and alone sufficient to explain demyelination in MS is still not understood.

In this perspective article we evaluate the possibility that a disruptive activation of the calpain-cathepsin axis in MS myelin can be reinforced by the activation of parallel Ca^{2+}-dependent aberrant events to induce primary myelin degeneration.

One that seems very relevant for MS is the cascade generated by Ca^{2+}-dependent mitochondrial dysfunctions (Nicholls, 2009). Mitochondria instability is not a new concept in MS pathology (Witte et al., 2014). In particular, Ca^{2+}-mediated aberrant events in the mitochondria, such as the opening of the mitochondria permeability transition pore (mPTP), trigger the release of intramembrane proteins (like cytochrome c) which, in turn, activate an apoptotic process named mitochondrial outer-membrane permeabilization (MOMP) (Ichas and Mazat, 1998).

Interestingly, cysteine protease activation and mitochondria pathological events share a number of common pathways. The activation of these pathways might instigate a "*vicious cycle*," strongly contributing to explain MS-related myelin degeneration. Here we propose that in MS, following excessive myelinic Ca^{2+} influx, calpain-cathepsin axis activation and MOMP play a synergic role in AMS destabilization and MAG degradation.

A LOOK AT THE COMPONENTS OF THE CYCLE: CYSTEINE PROTEASES, MITOCHONDRIA AND MAG DEGRADATION

Cysteine proteases actively promote protein catabolism inside the cellular compartment (Verma et al., 2016). Among them, calpain co-exists in two main isoforms: *calpain-1* (mu-type) and *calpain-2* (m-type, which requires higher Ca^{2+} concentrations to be activated) (Yamashima, 2004; Potz et al., 2016). Similarly, cathepsins are present in different forms (Turk et al., 2012).

Besides their role in cellular degeneration, some of these proteases seem particularly relevant for MS pathology. For instance, the L form of cathepsin is thought to play a role in MAG hydrolysis, truncating this molecule into a less functional version named dMAG (Stebbins et al., 1997). Notably, a selective MAG loss is spotted in the lesion formation in several neurodegenerative diseases including Kearns-Sayre syndrome (Lax et al., 2012) and MS (Aboul-Enein et al., 2003). Therefore, a dMAG formation operated by a calpain-cathepsin axis activation may hold important consequences for AMS stability, recapitulating the dynamic of AMS destabilization/myelin-axonal degeneration in MS.

Although the biochemical steps that lead to MAG degradation are not entirely understood (Paivalainen et al., 2003), studies have proposed that cathepsin-dependent MAG truncation may happen via different pathways. One implies a direct effect of cysteine proteases on MAG intracellular domains (Stebbins et al., 1997), while another mechanism operates via an indirect Ca^{2+}-dependent lysosomal fusion with the plasma membrane. The latter promotes cathepsin secretion through vesicular exocytosis (Rodriguez et al., 1997; Hashimoto et al., 2015). Finally, cathepsins are also shown to activate matrix metalloproteases (MMPs) (Milward et al., 2008). This third pathway may influence MAG stability through an extracellular matrix degradation and increase in myelin motility (Kroemer and Jaattela, 2005; Gu et al., 2015). Altered myelin motility may profoundly affect the ability of axon and myelin to establish a rigidly regulated point-to-point synapse (Micu et al., 2018), hampering the metabolic coupling they share (Beirowski et al., 2014; Micu et al., 2018; **Figure 1C**).

Mitochondria dysfunctionalities may corroborate the effects of these proteases on MS myelin. For instance, MOMP is shown to induce a cascade of deteriorating events such as those catalyzed by caspase-9 and−3 (Parsons and Green, 2010). Additionally, due to the role of mitochondria in Ca^{2+} storage, instability at the level of these organelles is able to instigate a detrimental extra-mitochondrial Ca^{2+} release (Montero et al., 2001). The latter effect might add up to the effects caused by augmented NMDAR-dependent myelinic Ca^{2+} influx.

On top of these processes, mitochondria respiratory chain defects are thought to cause MAG loss in relatively non-inflammatory environments. For instance, studies have shown

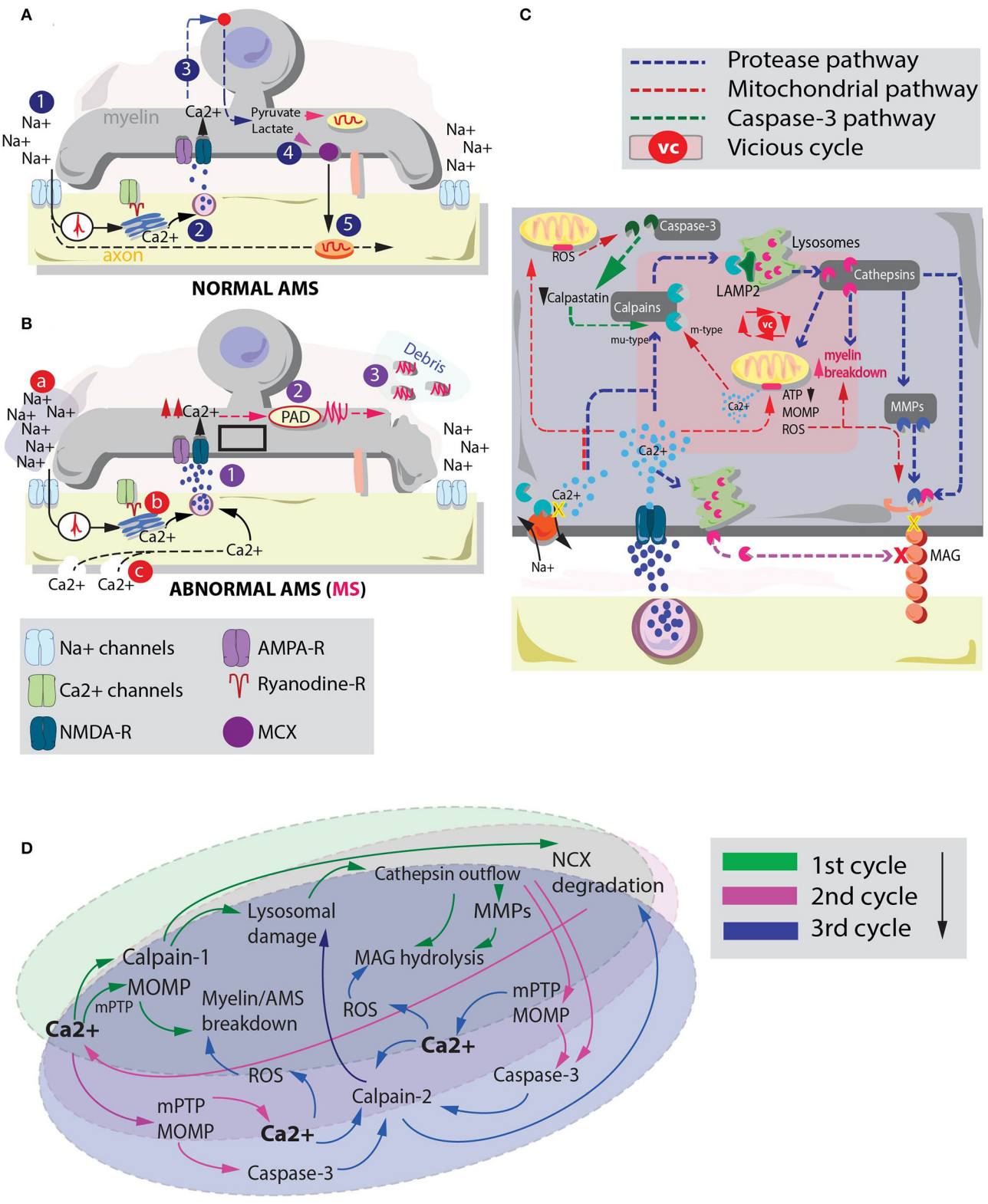

FIGURE 1 | Interplay between cysteine protease activation and mitochondrial deteriorating processes in MS myelin degeneration. **(A)** graphical depiction of the AMS in physiological conditions (Micu et al., 2018). The action potential (1) is sensed by Ca^{2+} channels which via a ryanodine receptor-dependent mechanism mobilize the

(Continued)

FIGURE 1 | axoplasmic Ca^{2+} reserves. Ca^{2+} triggers vesicle fusion and glutamate release (2). Glutamate activates NMDA-Rs promoting myelinic Ca^{2+} influx and glycolysis processes. This sequence of events leads to pyruvate and lactate production (3). Lactate is ultimately transported to the axon to metabolically assist axonal mitochondria. **(B)** In pathological conditions, excessive Ca^{2+} myelinic influx is promoted via altered stimulation of NMDA receptors (Micu et al., 2018). Possible players involved in such effect are: (a) an altered Na^{+} homeostasis, where augmented extracellular Na^{+} may lead to intra-axonal aberrant processes that promote Ca^{2+} influx (Craner et al., 2004); (b) altered Ca^{2+} mobilization inside the axoplasmic reticulum (Micu et al., 2018) and (c) the presence of subtle axonal nanoruptures which make axonal cylinder permeable to Ca^{2+} (Witte et al., 2019). Irrespective of the mechanism by which altered Ca^{2+} entrance in the myelin is triggered, such phenomenon (1) may lead to a consequent activation of PADs (2). PADs citrullinate myelin proteins (e.g., MBP). Myelin degenerative processes together with citrullination of MBP promote biochemically altered (highly immunogenic) debris formation (3). **(C)** Summary of the principal pathways by which cysteine proteases (blue pathway) and dysregulated mitochondria (red pathway) can affect myelin and MAG stability. These processes and their interplay may be responsible of myelin and axonal breakdown in MS. Pink box inside myelin depicts the players of a possible vicious cycle between cysteine protease activation and mitochondrial instability in MS-related myelin breakdown. **(D)** Schematic representation of a possible vicious cycle activated by enhanced myelinic Ca^{2+} entrance. Primary Ca^{2+} entrance instigates a parallel activation of mu-type-calpains (calpain-1) and mitochondria degenerative processes. Secondary Ca^{2+} accumulation due to Na^{+}/Ca^{2+} exchanger (NCX) degradation and Ca^{2+} efflux from mitochondria might take part in a tertiary activation of m-type calpains (calpain-2) which can exacerbate lysosomal and mitochondria instability leading to heightened myelin damage.

that MAG loss is associated with prominent nuclear expression of HIF-1α, a marker for hypoxia-like metabolic tissue injury. HIF-1α can be induced by either mitochondrial increase in intracellular reactive oxygen species (ROS) production (Aboul-Enein et al., 2003) or by impaired mitochondrial respiratory chain functions (Semenza, 2000). Finally, studies on Kearns-Sayre syndrome reported primary MAG loss and consequent demyelination (Lax et al., 2012). The onset of this disorder is thought to be due to primary mitochondrial respiratory chain defects as a result of a single mtDNA deletion. Therefore, it might be possible that also in MS mitochondrial instability instigates MAG loss with consequent induction of AMS instability/myelin degeneration.

DISCUSSION: THE VICIOUS CYCLE EXPLAINED

Aberrant myelin Ca^{2+} influx in the AMS may be an essential trigger in MS (Micu et al., 2018). Although a clear sequence of events is still ignored, Ca^{2+} dysregulations are a potent source of parallel biochemical processes that may synergistically partake in degenerative conditions. This interplay might generate a vicious cycle where Ca^{2+}-mediated events reinforce each other, explaining complex structural and biochemical alterations like those observed in MS brains. Interestingly, in the case of cysteine proteases and mitochondria dysfunctionality, both processes are crucially involved in degeneration/apoptosis (McConkey, 1998; Festjens et al., 2006; Zong and Thompson, 2006). Therefore, a combined activation of these pathways would likely explain the consequent highly immunogenic myelin fragmentation observed e.g., in cuprizone mouse models (Caprariello et al., 2018).

As one of the roles of the AMS is to supply the axonal compartment with lactate for metabolic purposes, a cysteine protease/mitochondrial-dependent myelin breakup can induce a secondary stage of axonal virtual hypoxia leading to axon disintegration (Stys et al., 2012; Micu et al., 2018).

Cysteine proteases and mitochondria might hypothetically interact in several ways to decide the fate of myelin in MS. Following an initial myelinic Ca^{2+} increase a primary co-activation of calpain-1 and mPTP/MOMP might occur (**Figures 1C,D**). These events may instigate lysosomal cathepsin outflow and primitive myelin breakdown, respectively (**Figure 1D**, 1st cycle). Elevated levels of cathepsins can either directly or indirectly affect MAG stability (Rodriguez et al., 1997; Stebbins et al., 1997; Milward et al., 2008; Hashimoto et al., 2015). Furthermore, simultaneously altered level of calpains can promote a cleavage of the Na^{+}/Ca^{2+} exchange pump (NCX) (McConkey, 1998; Zong and Thompson, 2006). The latter phenomenon facilitates an additional intracellular Ca^{2+} accumulation which together with the ability of cathepsin-L and D to activate caspase-3 (Li et al., 1997; Yamashima, 2000) and MOMP (Kroemer and Jaattela, 2005) might greatly reinforce the cysteine protease/mitochondria vicious cycle (**Figure 1D**, 2nd cycle). In fact, caspase-3 is thought to be able to cleave calpastatin, an endogenous calpain inhibitor (Porn-Ares et al., 1998; Yamashima, 2004), instigating a protracted permanence of calpains in the myelinic compartment. Cleavage of calpastatin together with mPTP-dependent mitochondria Ca^{2+} release (De Marchi et al., 2014) might promote the activation and accumulation of other forms of degenerative calpains, like calpain-2, which require Ca^{2+} in the mM range to be activated (Baudry and Bi, 2016). Finally, parallel mitochondria disruptions caused by Ca^{2+} overload can induce ROS enhancement (Festjens et al., 2006) strongly adding up to the ongoing AMS instability, myelin breakdown and MAG degradation (**Figure 1D**, 3rd cycle and beyond).

CONCLUDING REMARKS

In this opinion article we proposed a scenario where a primary altered AMS stability might explain myelin breakdown and axonal degeneration in MS via Ca^{2+}-mediated aberrant events (Micu et al., 2018). Possible instigators of such processes are the cysteine proteases and mitochondria dysfunctionalities. Here we posit that these two players reinforce each other leading to a possible vicious cycle which may hold important consequences for myelin stability. To confirm this hypothesis rigidly controlled experiments should define whether an actual interplay between the activation of the calpain-cathepsin axis and MOMP exists and, in this case, whether one pathway temporarily precedes the other one.

AUTHOR CONTRIBUTIONS

AP, GS, and AL: conceived the study. AP: performed the literature research. GS and JG: provided critical revision to the manuscript. AP and AL: wrote the manuscript with input from all the authors.

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REFERENCES

Aboul-Enein, F., Rauschka, H., Kornek, B., Stadelmann, C., Stefferl, A., Bruck, W., et al. (2003). Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. *J. Neuropathol. Exp. Neurol.* 62, 25–33. doi: 10.1093/jnen/62.1.25

Baraban, M., Koudelka, S., and Lyons, D. A. (2018). Ca (2+) activity signatures of myelin sheath formation and growth *in vivo*. *Nat. Neurosci.* 21, 19–23. doi: 10.1038/s41593-017-0040-x

Barnett, M. H., and Prineas, J. W. (2004). Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol.* 55, 458–468. doi: 10.1002/ana.20016

Baudry, M., and Bi, X. (2016). Calpain-1 and calpain-2: the yin and yang of synaptic plasticity and neurodegeneration. *Trends Neurosci.* 39, 235–245. doi: 10.1016/j.tins.2016.01.007

Beirowski, B., Babetto, E., Golden, J. P., Chen, Y. J., Yang, K., Gross, R. W., et al. (2014). Metabolic regulator LKB1 is crucial for Schwann cell-mediated axon maintenance. *Nat. Neurosci.* 17, 1351–1361. doi: 10.1038/nn.3809

Caprariello, A. V., Rogers, J. A., Morgan, M. L., Hoghooghi, V., Plemel, J. R., Koebel, A., et al. (2018). Biochemically altered myelin triggers autoimmune demyelination. *Proc. Natl. Acad. Sci. U.S.A.* 115, 5528–5533. doi: 10.1073/pnas.1721115115

Chitnis, T. (2007). The role of CD4 T cells in the pathogenesis of multiple sclerosis. *Int. Rev. Neurobiol.* 79, 43–72. doi: 10.1016/S0074-7742(07)79003-7

Craner, M. J., Hains, B. C., Lo, A. C., Black, J. A., and Waxman, S. G. (2004). Co-localization of sodium channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE. *Brain* 127, 294–303. doi: 10.1093/brain/awh032

De Marchi, E., Bonora, M., Giorgi, C., and Pinton, P. (2014). The mitochondrial permeability transition pore is a dispensable element for mitochondrial calcium efflux. *Cell Calcium* 56, 1–13. doi: 10.1016/j.ceca.2014.03.004

Festjens, N., Vanden Berghe, T., and Vandenabeele, P. (2006). Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. *Biochim. Biophys. Acta* 1757, 1371–1387. doi: 10.1016/j.bbabio.2006.06.014

Gu, Y. H., Kanazawa, M., Hung, S. Y., Wang, X., Fukuda, S., Koziol, J. A., et al. (2015). Cathepsin L acutely alters microvessel integrity within the neurovascular unit during focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* 35, 1888–1900. doi: 10.1038/jcbfm.2015.170

Hashimoto, Y., Kondo, C., and Katunuma, N. (2015). An active 32-kDa cathepsin L is secreted directly from HT 1080 fibrosarcoma cells and not via lysosomal exocytosis. *PLoS ONE* 10:e0145067. doi: 10.1371/journal.pone.0145067

Henderson, A. P., Barnett, M. H., Parratt, J. D., and Prineas, J. W. (2009). Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann. Neurol.* 66, 739–753. doi: 10.1002/ana.21800

Ichas, F., and Mazat, J. P. (1998). From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. *Switching from low- to high-conductance state. Biochim. Biophys. Acta* 1366, 33–50. doi: 10.1016/S0005-2728(98)00119-4

Inglese, M., Madelin, G., Oesingmann, N., Babb, J. S., Wu, W., Stoeckel, B., et al. (2010). Brain tissue sodium concentration in multiple sclerosis: a sodium imaging study at 3 tesla. *Brain* 133, 847–857. doi: 10.1093/brain/awp334

Kroemer, G., and Jaattela, M. (2005). Lysosomes and autophagy in cell death control. *Nat. Rev. Cancer* 5, 886–897. doi: 10.1038/nrc1738

Lassmann, H., and Ransohoff, R. M. (2004). The CD4-Th1 model for multiple sclerosis: a critical [correction of crucial] re-appraisal. *Trends Immunol.* 25, 132–137. doi: 10.1016/j.it.2004.01.007

Lax, N. Z., Campbell, G. R., Reeve, A. K., Ohno, N., Zambonin, J., Blakely, E. L., et al. (2012). Loss of myelin-associated glycoprotein in kearns-sayre syndrome. *Arch. Neurol.* 69, 490–499. doi: 10.1001/archneurol.2011.2167

Leist, M., and Jaattela, M. (2001). Triggering of apoptosis by cathepsins. *Cell Death Differ.* 8, 324–326. doi: 10.1038/sj.cdd.4400859

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., et al. (1997). Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91, 479–489. doi: 10.1016/S0092-8674(00)80434-1

Llorens, F., Thune, K., Sikorska, B., Schmitz, M., Tahir, W., Fernandez-Borges, N., et al. (2017). Altered Ca(2+) homeostasis induces calpain-cathepsin axis activation in sporadic creutzfeldt-jakob disease. *Acta Neuropathol. Commun.* 5:35. doi: 10.1186/s40478-017-0431-y

McConkey, D. J. (1998). Biochemical determinants of apoptosis and necrosis. *Toxicol. Lett.* 99, 157–168. doi: 10.1016/S0378-4274(98)00155-6

Micu, I., Plemel, J. R., Caprariello, A. V., Nave, K. A., and Stys, P. K. (2018). Axo-myelinic neurotransmission: a novel mode of cell signalling in the central nervous system. *Nat. Rev. Neurosci.* 19, 49–58. doi: 10.1038/nrn.2017.128

Milward, E., Kim, K. J., Szklarczyk, A., Nguyen, T., Melli, G., Nayak, M., et al. (2008). Cleavage of myelin associated glycoprotein by matrix metalloproteinases. *J. Neuroimmunol.* 193, 140–148. doi: 10.1016/j.jneuroim.2007.11.001

Miyazawa, T., Matsumoto, K., Ohmichi, H., Katoh, H., Yamashima, T., and Nakamura, T. (1998). Protection of hippocampal neurons from ischemia-induced delayed neuronal death by hepatocyte growth factor: a novel neurotrophic factor. *J. Cereb. Blood Flow Metab.* 18, 345–348. doi: 10.1097/00004647-199804000-00001

Montero, M., Alonso, M. T., Albillos, A., Garcia-Sancho, J., and Alvarez, J. (2001). Mitochondrial Ca(2+)-induced Ca(2+) release mediated by the Ca(2+) uniporter. *Mol. Biol. Cell.* 12, 63–71. doi: 10.1091/mbc.12.1.63

Nicholls, D. G. (2009). Mitochondrial calcium function and dysfunction in the central nervous system. *Biochim. Biophys. Acta* 1787, 1416–1424. doi: 10.1016/j.bbabio.2009.03.010

Paivalainen, S., Suokas, M., Lahti, O., and Heape, A. M. (2003). Degraded myelin-associated glycoprotein (dMAG) formation from pure human brain myelin-associated glycoprotein (MAG) is not mediated by calpain or cathepsin L-like activities. *J. Neurochem.* 84, 533–545. doi: 10.1046/j.1471-4159.2003.01539.x

Parsons, M. J., and Green, D. R. (2010). Mitochondria in cell death. *Essays Biochem.* 47, 99–114. doi: 10.1042/bse0470099

Porn-Ares, M. I., Samali, A., and Orrenius, S. (1998). Cleavage of the calpain inhibitor, calpastatin, during apoptosis. *Cell Death Differ.* 5, 1028–1033. doi: 10.1038/sj.cdd.4400424

Potz, B. A., Abid, M. R., and Sellke, F. W. (2016). Role of calpain in pathogenesis of human disease processes. *J. Nat. Sci.* 2:e218.

Pronker, M. F., Lemstra, S., Snijder, J., Heck, A. J., Thies-Weesie, D. M., Pasterkamp, R. J., et al. (2016). Structural basis of myelin-associated glycoprotein adhesion and signalling. *Nat. Commun.* 7:13584. doi: 10.1038/ncomms13584

Ringold, S., Lynm, C., and Glass, R. M. (2005). JAMA patient page. *Multiple sclerosis. JAMA* 293:514. doi: 10.1001/jama.293.4.514

Rodriguez, A., Webster, P., Ortego, J., and Andrews, N. W. (1997). Lysosomes behave as Ca2+-regulated exocytic vesicles in fibroblasts and epithelial cells. *J. Cell. Biol.* 137, 93–104. doi: 10.1083/jcb.137.1.93

Semenza, G. L. (2000). Surviving ischemia: adaptive responses mediated by hypoxia-inducible factor 1. *J. Clin. Invest.* 106, 809–812. doi: 10.1172/JCI11223

Stebbins, J. W., Jaffe, H., Fales, H. M., and Moller, J. R. (1997). Determination of a native proteolytic site in myelin-associated glycoprotein. *Biochemistry* 36, 2221–2226. doi: 10.1021/bi962385x

Stys, P. K., Zamponi, G. W., Van Minnen, J., and Geurts, J. J. (2012). Will the real multiple sclerosis please stand up? *Nat. Rev. Neurosci.* 13, 507–514. doi: 10.1038/nrn3275

Trapp, B. D., and Quarles, R. H. (1982). Presence of the myelin-associated glycoprotein correlates with alterations in the periodicity of peripheral myelin. *J. Cell. Biol.* 92, 877–882. doi: 10.1083/jcb.92.3.877

Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T., Turk, B., et al. (2012). Cysteine cathepsins: from structure, function and regulation to new frontiers. *Biochim. Biophys. Acta* 1824, 68–88. doi: 10.1016/j.bbapap.2011.10.002

Verma, S., Dixit, R., and Pandey, K. C. (2016). Cysteine proteases: modes of activation and future prospects as pharmacological targets. *Front. Pharmacol.* 7:107. doi: 10.3389/fphar.2016.00107

Villalpando Rodriguez, G. E., and Torriglia, A. (2013). Calpain 1 induce lysosomal permeabilization by cleavage of lysosomal associated membrane protein 2. *Biochim. Biophys. Acta* 1833, 2244–2253. doi: 10.1016/j.bbamcr.2013.05.019

Witte, M. E., Mahad, D. J., Lassmann, H., and Van Horssen, J. (2014). Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. *Trends Mol. Med.* 20, 179–187. doi: 10.1016/j.molmed.2013.11.007

Witte, M. E., Schumacher, A. M., Mahler, C. F., Bewersdorf, J. P., Lehmitz, J., Scheiter, A., et al. (2019). Calcium influx through plasma-membrane nanoruptures drives axon degeneration in a model of multiple sclerosis. *Neuron 101*, 615–624.e615. doi: 10.1016/j.neuron.2018.12.023

Yamashima, T. (2000). Implication of cysteine proteases calpain, cathepsin and caspase in ischemic neuronal death of primates. *Prog. Neurobiol.* 62, 273–295. doi: 10.1016/S0301-0082(00)00006-X

Yamashima, T. (2004). Ca2+-dependent proteases in ischemic neuronal death: a conserved 'calpain-cathepsin cascade' from nematodes to primates. *Cell Calcium* 36, 285–293. doi: 10.1016/j.ceca.2004.03.001

Zong, W. X., and Thompson, C. B. (2006). Necrotic death as a cell fate. *Genes Dev.* 20, 1–15. doi: 10.1101/gad.1376506

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Involvement of Genetic Factors in Multiple Sclerosis

Laura Ferrè [1,2,3,4], *Massimo Filippi* [1,2,4,5,6] *and Federica Esposito* [1,2,3]*

[1] *Neurology Unit, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy,* [2] *Neurorehabilitation Unit, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy,* [3] *Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology, Division of Neuroscience, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy,* [4] *Vita-Salute San Raffaele University, Milan, Italy,* [5] *Neurophysiology Unit, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy,* [6] *Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy*

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INTRODUCTION

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS) that affects young adults between 20 and 40 years of age, with a higher prevalence in females (female-to-male ratio 3:1) (Dilokthornsakul et al., 2016). Even though its etiology is still debated, it has historically been considered an immune-mediated disease, whose histopathological hallmarks are inflammation, demyelination, and neurodegeneration (Frohman et al., 2006; Lassmann, 2018). Indeed, acute demyelinating lesions characterized by blood brain barrier breakdown, lymphocyte infiltration, oligodendrocyte loss and astrocytic activation, mainly located in myelin-rich white-matter areas, are distinctive of the disease. Nonetheless, in more recent years it became clear that axonal loss and primary demyelination in the absence of acute inflammatory infiltrates are also present since the earliest stages of the disease (Henderson et al., 2009), challenging the idea that neurodegeneration is secondary to CNS inflammation (Louapre and Lubetzki, 2015). As a matter of fact, it is still unclear whether neurodegeneration is a direct consequence of inflammatory CNS injury or whether it represents a primitive independent process, and a better understanding of the interplay between these two aspects of the disease is warranted. In this perspective, studies investigating genetic factors associated with MS onset and progression can help to shed light on this matter; in fact, compared to cross-sectional histopathological studies that do not permit to establish temporal and/or causal relationship, genetic association studies allow to infer causality and to pinpoint molecular pathways and cell types that play a key role in MS pathogenesis.

HLA ASSOCIATION

The first MS-associated genetic risk locus discovered in 1972 was located in the human leukocyte antigen (HLA) class I region on chromosome 6 (Jersild et al., 1972; Naito et al., 1972). Since then, several HLA class I and II alleles have been associated with an increased risk of developing MS (Hauser et al., 1989; Patsopoulos et al., 2013) or were found to be protective (Fogdell-Hahn et al., 2000).

The strongest association has been demonstrated with the HLA DRB1*1501 allele (Hauser et al., 1989; Oksenberg et al., 2004; Patsopoulos et al., 2013) that confers an almost 3-fold increased risk of MS. The most recent genome-wide association study (GWAS) in MS (Patsopoulos et al., 2019) identified up to 32 independent MS risk-variants in the major histocompatibility complex (MHC) region and, overall, the MHC locus alone is estimated to explain 20% of MS heritability (Patsopoulos et al., 2019).

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Bert A. 'T Hart,
University Medical Center Groningen, Netherlands

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Giovanni Ristori,
Sapienza University of Rome, Italy

***Correspondence:**
Federica Esposito
esposito.federica@hsr.it

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The HLA locus maps on chromosome 6p21.3 and encompasses more than 200 genes, with important roles in maturation and regulation of the T cell compartment as well as in other immunological processes, supporting the hypothesis that MS is an immune-mediated disease arising from a dysregulation of the peripheral immune system which targets the CNS. Consistently, a fine-mapping study of the MHC region (Patsopoulos et al., 2013) has shown that some of the MS-associated variants in this locus affect the amino-acidic sequence of the peptide-binding groove, potentially influencing antigen recognition and T-cell repertoire specificity.

GENOME-WIDE ASSOCIATION STUDIES OF MS SUSCEPTIBILITY

Since 1970s, the knowledge of MS genetic architecture has extraordinarily advanced and several GWAS have been performed, identifying hundreds of additional MS genetic risk variants outside the MHC locus (The International Multiple Sclerosis Genetics Consortium et al., 2007; Bahlo et al., 2009; Baranzini et al., 2009; Sanna et al., 2010; Patsopoulos et al., 2011, 2019; Sawcer et al., 2011; Beecham et al., 2013). GWAS are particularly helpful in the discovery of novel disease variants, because they do not depend on "a priori" assumption on disease pathogenesis and can help to unravel novel molecular mechanisms underlying the disease.

The first MS GWAS (The International Multiple Sclerosis Genetics Consortium et al., 2007) was performed on 931 family trios and identified variants in the interleukin-7 receptor (IL7R) and in the interleukin-2 receptor (IL2RA) loci (Gregory et al., 2007; Weber et al., 2008), again pointing to the crucial role played by the immune system. In the following years, the collaborative efforts of the International MS Genetic Consortium (IMSGC) allowed to combine MS cohorts from several countries, improving the statistical power and leading to a remarkable increase in the number of the identified MS risk variants. In 2011, the analysis of 9,772 MS cases and 17,376 controls from 15 different countries, followed by a replication step in 4,218 cases and 7,296 healthy controls (Sawcer et al., 2011), allowed to confirm most of the previously reported associations and to detect 29 novel risk variants. Noteworthy, the prioritized effects were enriched in genes involved in lymphocyte functions and in particular in T cell activation and proliferation. Moreover, genes linked to Vitamin D metabolism, such as CYP27B1, CYP24A1, and genes coding for targets of MS immune-modulatory therapies, including VCAM1 for natalizumab and IL2RA for daclizumab, were also highlighted. More recently, the greatest advancement in MS genetics field was made possible by a meta-analysis involving 47,351 MS subjects and 68,284 healthy controls (Patsopoulos et al., 2019) that increased the number of MS-associated variants up to 200 autosomal SNPs outside the MHC region and one variant on the X chromosome. Once again, the identified variants seem to be enriched in loci which are active in immune cells, both from the adaptive (T and B cells) and the innate (natural killer and dendritic cells) immune compartments; conversely there was no enrichment of MS susceptibility loci in CNS tissues and, also when analyzing induced pluripotent stem cell derived neurons and purified human astrocytes or microglia, the only significant enrichment was detected in microglial cells, suggesting that also CNS resident immune compartment could play a role in the disease. Similarly, when assessing the effect of the selected variants on gene expression modulation in specific tissues (eQTL effect) and when evaluating the biological pathways enriched in MS genes, findings were indicative of the central role of immune cells, while putative functional consequences in neurons and other CNS cells need further investigations. A more recent study (Factor et al., 2020) applied a so-called "outside variant approach" to identify additional risk variants that physically interact with MS-associated genes and to pinpoint cell-types in which these genes exert their pathogenic role. As expected, the majority of MS loci were predicted to act in T cells, but also B cells and myeloid cells were involved, underlining the role of both the innate and adaptive immune system. Moreover, the authors also identified 6 loci that were predicted to act in the CNS and they hypothesized that a dysregulation of transcriptional elongation in oligodendrocytes could contribute to MS pathogenesis.

Finally, the main role played by the immune system is also reflected in the significant proportion of MS risk variants that is shared with other autoimmune diseases (Richard-Miceli and Criswell, 2012), such as type 1 diabetes, rheumatoid arthritis and intestinal bowel diseases, even though sometimes with opposite effect. The common background with other immunological conditions seems to imply the existence of a genetic basis predisposing to peripheral immune dysregulation; other risk factors, such as environmental exposures and/or infections, could then play a role in determining the target tissue and phenotypic expression of this autoimmune predisposition.

THE ROLE OF LOW-FREQUENCY/RARE VARIANTS

Classic GWAS take advantage of very large numbers of enrolled subjects to screen hundreds of thousands common variants over the entire genome but are largely underpowered to detect association with low-frequency and rare variants. More recently, some studies investigated the contribution of rare variants in MS heritability using a candidate gene approach or a whole exome analysis in sporadic and familiar MS cases. Specifically, a study conducted in about 68,000 subjects identified four novel variants driving MS risk independently of common-variant signals, which are implicated in regulatory T cell homeostasis and regulation, IFN gamma biology, and NFkB signaling (Mitrovič et al., 2018). Another study identified an increased burden of rare variants with high predicted pathogenicity in MS cases compared to controls when analyzing genes of the inflammasome pathway (e.g., NLRP1/3, CASP1) (Vidmar et al., 2019). Similarly, an additional work on 34 multi-incident MS families also identified some putative risk variants implicating the inflammasome pathway (NLRP12), as well as other biological processes involved

in innate immunological responses, again stressing the role of immune factors in the disease (Vilariño-Güell et al., 2019).

GENETIC FACTORS INFLUENCING DISEASE SEVERITY

Overall, genetic studies on MS susceptibility seem to denote a major role for immune-related processes in disease pathogenesis; however, these studies do not take into consideration the highly variable degree of neurodegeneration and disability accumulation that characterize the disease. For this reason, genetic studies investigating variants associated with disease severity represent a complementary approach in understanding MS pathological basis.

First of all, candidate gene studies have assessed the role of genes implicated in MS susceptibility in determining disease course, failing to identify any significant association (Jensen et al., 2010) except for the HLA-DRB1*1501 allele (Hauser et al., 2000; Barcellos et al., 2003). Interestingly enough, a subsequent GWAS (Baranzini et al., 2009) also failed to identify an enrichment of associated genes implicated in immunological functions. On the contrary, when disease severity was assessed using MRI measures such as brain volume and T2 lesion load, there was an over-representation of genes related to neural processes (e.g., GRIN2A, NLGN1) such as glutamate signaling and axon guidance. Similarly, a network analysis on results from a GWAS that used brain glutamate concentrations as endophenotype (Baranzini et al., 2010) identified a module enriched in genes implicated in glutamate biology that correlated with the level of brain atrophy and N-Acetylaspartate decline over time, pointing to a possible role of excitotoxicity in modulating disease severity.

Furthermore, an additional GWAS (Briggs et al., 2011) that investigated variants associated with disease severity using the MS severity score (MSSS) and in which a pathway analysis was applied showed an enrichment of genes involved in axon guidance and signaling and neuronal processes as well as with interferon α/β receptor binding and antigen processing and presentation.

PHARMACOGENETIC STUDIES

Finally, also works investigating genetic determinants of response to disease modifying treatments can add to the knowledge of the biological basis of the disease.

Interferonβ (IFNβ) was the first drug to be approved for MS and is also the most investigated in pharmacogenomic studies; specifically, the first of these studies focused on genes known to be implicated in its mechanism of action applying a candidate gene approach. At least 15 genes associated with IFNβ response were identified (Cunningham et al., 2005; Martínez et al., 2006; Gross et al., 2011; Kulakova et al., 2012), although not all the associations have been validated in independent cohorts. Not surprisingly, associated polymorphisms mapped to region encoding type I IFN receptor (Cunningham et al., 2005), IFN response-element sequences, IFN regulatory transcription factors (Gross et al., 2011) and to loci encoding different cytokine genes (IL10, IFNγ) (Martínez et al., 2006; Kulakova et al., 2012). Candidate studies also evaluated the role of the HLA class II locus, and mainly of the HLA DR2 haplotype, in influencing IFNβ response, but no significant association was found (Gross et al., 2011).

More interesting were the results of GWAS on response to IFNβ (Byun et al., 2008; Comabella et al., 2009; Esposito et al., 2015; Clarelli et al., 2017; Mahurkar et al., 2017); these investigations not only confirmed the role of genes implicated in the IFNβ pathway, such as IFNAR2, but also identified genes involved in neuronal functions like GPC5 (Byun et al., 2008), the glutamate receptors GRIA3 (Comabella et al., 2009), GRM3 and GRIK2 (Clarelli et al., 2017) and SLC9A9 (Esposito et al., 2015), which has been linked to neuronal excitability (Gu et al., 2001), pointing to a possible role for glutamate metabolism and excitotoxicity in modulating drug response. Similarly, candidate gene studies on response to glatiramer acetate (GA) (Fusco et al., 2001; Grossman et al., 2007; Tsareva et al., 2012; Kulakova et al., 2017) focused mainly on immune-related genes and found that the clinical outcome during GA treatment is associated with HLA class II genes (Fusco et al., 2001; Gross et al., 2011) and other genes involved in T cell activation (TCRB, Grossman et al., 2007), antigen presentation (CD86) or proinflammatory signaling [(IL1R1 and IL12RB2, Grossman et al., 2007), (CCR5, Tsareva et al., 2012)]. Additionally, a recent GWAS on more than 2,500 GA-treated MS patients (Ross et al., 2017) selected a set of 4 SNPs mapping to HLA-DQB2, MBP, UVRAG, and ZAK, which distinguished signature-positive or negative patients, signature-positive subjects displaying a better outcome over signature-negative individuals.

DISCUSSION

The results of genetic studies on MS susceptibility (The International Multiple Sclerosis Genetics Consortium et al., 2007; Patsopoulos et al., 2019) identified a strong enrichment of genes and biological processes implicated in immune functions, suggesting that a dysregulation of immune responses, promoted by the genetic background and potentially triggered by environmental factors, is the main mechanism of the disease. Overall, apart from a single study that predicted 6 MS risk loci to be mainly active in CNS cells, where they can disrupt oligodendrocyte maturation (Factor et al., 2020), no particular enrichment in genes and pathways involved in CNS development and regulation was found, implying that neurodegeneration is more likely to derive from an inflammatory insult than to be a primary process itself. However, these studies mainly enrolled patients with a relapsing-remitting form of the disease, which is characterized by a high level of inflammatory activity. Thus, it is possible that genetic variants affecting the degree of neuronal damage and disability accumulation were not adequately picked-up by such studies. Indeed, genetic studies that focused on disease severity showed genes involved in neuronal processes, and in particular in glutamate biology, as associated with the degree of disability progression and neurodegeneration (Baranzini et al.,

2009, 2010). Noteworthy, some of these genes and pathways were also associated with response to MS treatments (Comabella et al., 2009; Esposito et al., 2015; Clarelli et al., 2017). Unfortunately, most of the genetic studies on MS severity and treatment response have been performed in relatively modest datasets and lack a validation in larger, independent cohorts due to the difficulty in obtaining detailed longitudinal clinical information in huge cohorts. Nonetheless, these results seem to suggest that inflammation is not the only determinant of disease course and also neuronal factors can modulate the level of neurodegeneration and disability progression in MS. Further studies on larger datasets are required to validate these results and to help identifying potential therapeutic targets to halt neurodegeneration in MS.

AUTHOR CONTRIBUTIONS

LF: design and conceptualization of the study and manuscript writing. MF: revision of the manuscript for intellectual content. FE: conceptualization of the study and revision of the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

REFERENCES

Bahlo, M., Booth, D. R., Simon, A., Broadley, B., Foote, S. J., Griffiths, L. R., et al. (2009). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat. Genet.* 41, 824–828. doi: 10.1038/ng.396

Baranzini, S. E., Srinivasan, R., Khankhanian, P., Okuda, D. T., Nelson, S. J., Matthews, P. M., et al. (2010). Genetic variation influences glutamate concentrations in brains of patients with multiple sclerosis. *Brain* 133, 2603–2611. doi: 10.1093/brain/awq192

Baranzini, S. E., Wang, J., Gibson, R. A., Galwey, N., Naegelin, Y., Barkhof, F., et al. (2009). Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum. Mol. Genet.* 18, 767–778. doi: 10.1093/hmg/ddn388

Barcellos, L. F., Oksenberg, J. R., Begovich, A. B., Martin, E. R., Schmidt, S., Vittinghoff, E., et al. (2003). HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. *Am. J. Hum. Genet.* 72, 710–716. doi: 10.1086/367781

Beecham, A. H., Patsopoulos, N. A., Xifara, D. K., Davis, M. F., Kemppinen, A., Cotsapas, C., et al. (2013). Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat. Genet.* 45, 1353–1360. doi: 10.1038/ng.2770

Briggs, F. B. S., Shao, X., Goldstein, B. A., Oksenberg, J. R., Barcellos, L. F., and De Jager, P. L. (2011). Genome-wide association study of severity in multiple sclerosis. *Genes Immun.* 12, 615–625. doi: 10.1038/gene.2011.34

Byun, E., Caillier, S. J., Montalban, X., Villoslada, P., Fernández, O., Brassat, D., et al. (2008). Genome-wide pharmacogenomic analysis of the response to interferon beta therapy in multiple sclerosis. *Arch. Neurol.* 65, 337–344. doi: 10.1001/archneurol.2008.47

Clarelli, F., Liberatore, G., Sorosina, M., Osiceanu, A. M., Esposito, F., Mascia, E., et al. (2017). Pharmacogenetic study of long-term response to interferon-β treatment in multiple sclerosis. *Pharmacogenomics J.* 17, 84–91. doi: 10.1038/tpj.2015.85

Comabella, M., Craig, D. W., Morcillo-Suárez, C., Río, J., Navarro, A., Fernández, M., et al. (2009). Genome-wide scan of 500 000 single-nucleotide polymorphisms among responders and nonresponders to interferon beta therapy in multiple sclerosis. *Arch. Neurol.* 66, 972–978. doi: 10.1001/archneurol.2009.150

Cunningham, S., Graham, C., Hutchinson, M., Droogan, A., O'Rourke, K., Patterson, C., et al. (2005). Pharmacogenomics of responsiveness to interferon IFN-beta treatment in multiple sclerosis: a genetic screen of 100 type I interferon-inducible genes. *Clin. Pharmacol. Ther.* 78, 635–646. doi: 10.1016/j.clpt.2005.08.018

Dilokthornsakul, P., Valuck, R. J., Nair, K. V., Corboy, J. R., Allen, R. R., and Campbell, J. D. (2016). Multiple sclerosis prevalence in the United States commercially insured population. *Neurology* 86, 1014–1021. doi: 10.1212/WNL.0000000000002469

Esposito, F., Sorosina, M., Ottoboni, L., Lim, E. T., Replogle, J. M., Raj, T., et al. (2015). A pharmacogenetic study implicates SLC9a9 in multiple sclerosis disease activity. *Ann. Neurol.* 78, 115–127. doi: 10.1002/ana.24429

Factor, D. C., Barbeau, A. M., Allan, K. C., Hu, L. R., Madhavan, M., Hoang, A. T., et al. (2020). Cell type-specific intralocus interactions reveal oligodendrocyte mechanisms in MS. *Cell* 181, 382–395.e21. doi: 10.1016/j.cell.2020.03.002

Fogdell-Hahn, A., Ligers, A., Grønning, M., Hillert, J., and Olerup, O. (2000). Multiple sclerosis: a modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 55, 140–148. doi: 10.1034/j.1399-0039.2000.550205.x

Frohman, E. M., Racke, M. K., and Raine, C. S. (2006). Multiple sclerosis–the plaque and its pathogenesis. *N. Engl. J. Med.* 354, 942–955. doi: 10.1056/NEJMra052130

Fusco, C., Andreone, V., Coppola, G., Luongo, V., Guerini, F., Pace, E., et al. (2001). HLA-DRB1*1501 and response to copolymer-1 therapy in relapsing-remitting multiple sclerosis. *Neurology* 57, 1976–1979. doi: 10.1212/WNL.57.11.1976

Gregory, S. G., Schmidt, S., Seth, P., Oksenberg, J. R., Hart, J., Prokop, A., et al. (2007). Interleukin 7 receptor α chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat. Genet.* 39, 1083–1091. doi: 10.1038/ng2103

Gross, R., Healy, B. C., Cepok, S., Chitnis, T., Khoury, S. J., Hemmer, B., et al. (2011). Population structure and HLA DRB1 1501 in the response of subjects with multiple sclerosis to first-line treatments. *J. Neuroimmunol.* 233, 168–174. doi: 10.1016/j.jneuroim.2010.10.038

Grossman, I., Avidan, N., Singer, C., Goldstaub, D., Hayardeny, L., Eyal, E., et al. (2007). Pharmacogenetics of glatiramer acetate therapy for multiple sclerosis reveals drug-response markers. *Pharmacogenet. Genomics* 17, 657–666. doi: 10.1097/FPC.0b013e3281299169

Gu, X. Q., Yao, H., and Haddad, G. G. (2001). Increased neuronal excitability and seizures in the Na + /H + exchanger null mutant mouse. *Am. J. Physiol. Cell Physiol.* 281, C496–C503. doi: 10.1152/ajpcell.2001.281.2.C496

Hauser, S. L., Fleischnick, E., Weiner, H. L., Marcus, D., Awdeh, Z., Yunis, E. J., et al. (1989). Extended major histocompatibility complex haplotypes in patients with multiple sclerosis. *Neurology* 39, 275–277. doi: 10.1212/WNL.39.2.275

Hauser, S. L., Oksenberg, J. R., Lincoln, R., Garovoy, J., Beck, R. W., Cole, S. R., et al. (2000). Interaction between HLA-DR2 and abnormal brain MRI in optic neuritis and early MS. *Neurology* 54, 1859–1861. doi: 10.1212/WNL.54.9.1859

Henderson, A. P. D., Barnett, M. H., Parratt, J. D. E., and Prineas, J. W. (2009). Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann. Neurol.* 66, 739–753. doi: 10.1002/ana.21800

Jensen, C. J., Stankovich, J., Van der Walt, A., Bahlo, M., Taylor, B. V., van der Mei, I. A. F., et al. (2010). Multiple sclerosis susceptibility-associated SNPs do not influence disease severity measures in a cohort of Australian MS patients. *PLoS ONE* 5:e10003. doi: 10.1371/journal.pone.0010003

Jersild, C., Svejgaard, A., and Fog, T. (1972). HL-A antigens and multiple sclerosis. *Lancet* 1, 1240–1241. doi: 10.1016/S0140-6736(72)90962-2

Kulakova, O., Bashinskaya, V., Kiselev, I., Baulina, N., Tsareva, E., Nikolaev, R., et al. (2017). Pharmacogenetics of glatiramer acetate therapy for multiple sclerosis: the impact of genome-wide association studies identified disease risk loci. *Pharmacogenomics* 18, 1563–1574. doi: 10.2217/pgs-2017-0058

Kulakova, O. G., Tsareva, E. Y., Boyko, A. N., Shchur, S. G., Gusev, E. I., Lvovs, D., et al. (2012). Allelic combinations of immune-response genes as possible composite markers of IFN-β efficacy in multiple sclerosis patients. *Pharmacogenomics* 13, 1689–1700. doi: 10.2217/pgs.12.161

Lassmann, H. (2018). Multiple sclerosis pathology. *Cold Spring Harb. Perspect. Med*. 8:a028936. doi: 10.1101/cshperspect.a028936

Louapre, C., and Lubetzki, C. (2015). Neurodegeneration in multiple sclerosis is a process separate from inflammation: Yes. *Mult. Scler*. 21, 1626–1628. doi: 10.1177/1352458515587598

Mahurkar, S., Moldovan, M., Suppiah, V., Sorosina, M., Clarelli, F., Liberatore, G., et al. (2017). Response to interferon-beta treatment in multiple sclerosis patients: a genome-wide association study. *Pharmacogenomics J*. 17, 312–318. doi: 10.1038/tpj.2016.20

Martínez, A., de las Heras, V., Mas Fontao, A., Bartolom,é, M., de la Concha, E. G., Urcelay, E., et al. (2006). An IFNG polymorphism is associated with interferon-beta response in Spanish MS patients. *J. Neuroimmunol*. 173, 196–199. doi: 10.1016/j.jneuroim.2005.12.002

Mitrovič, M., Patsopoulos, N. A., Beecham, A. H., Dankowski, T., Goris, A., Dubois, B., et al. (2018). Low-frequency and rare-coding variation contributes to multiple sclerosis risk. *Cell* 175, 1679–1687.e7. doi: 10.1016/j.cell.2018.09.049

Naito, S., Namerow, N., Mickey, M. R., and Terasaki, P. I. (1972). Multiple sclerosis: association with HL-A3. *Tissue Antigens* 2, 1–4.

Oksenberg, J. R., Barcellos, L. F., Cree, B. A. C., Baranzini, S. E., Bugawan, T. L., Khan, O., et al. (2004). Mapping multiple sclerosis susceptibility to the HLA-DR locus in African Americans. *Am. J. Hum. Genet*. 74, 160–167. doi: 10.1086/380997

Patsopoulos, N. A., Baranzini, S. E., Santaniello, A., Shoostari, P., Cotsapas, C., Wong, G., et al. (2019). Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science* 365:eaav7188. doi: 10.1126/science.aav7188

Patsopoulos, N. A., Barcellos, L. F., Hintzen, R. Q., Schaefer, C., van Duijn, C. M., Noble, J. A., et al. (2013). Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. *PLoS Genet*. 9:e1003926. doi: 10.1371/journal.pgen.1003926

Patsopoulos, N. A., Esposito, F., Reischl, J., Lehr, S., Bauer, D., Heubach, J., et al. (2011). Genome-wide meta-analysis identifies novel multiple sclerosis susceptibility loci. *Ann. Neurol*. 70, 897–912. doi: 10.1002/ana.22609

Richard-Miceli, C., and Criswell, L. A. (2012). Emerging patterns of genetic overlap across autoimmune disorders. *Genome Med*. 4:6. doi: 10.1186/gm305

Ross, C. J., Towfic, F., Shankar, J., Laifenfeld, D., Thoma, M., Davis, M., et al. (2017). A pharmacogenetic signature of high response to copaxone in late-phase clinical-trial cohorts of multiple sclerosis. *Genome Med*. 9:50. doi: 10.1186/s13073-017-0436-y

Sanna, S., Pitzalis, M., Zoledziewska, M., Zara, I., Sidore, C., Murru, R., et al. (2010). Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. *Nat. Genet*. 42, 495–497. doi: 10.1038/ng.584

Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C. A., Patsopoulos, N. A., Moutsianas, L., et al. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219. doi: 10.1038/nature10251

The International Multiple Sclerosis Genetics Consortium, Hafler, D. A., Compston, A., Sawcer, S., Lander, E. S., Daly, M. J., et al. (2007). Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med*. 357, 851–862. doi: 10.1056/NEJMoa073493

Tsareva, E. Y., Kulakova, O. G., Boyko, A. N., Shchur, S. G., Lvovs, D., Favorov, A. V., et al. (2012). Allelic combinations of immune-response genes associated with glatiramer acetate treatment response in Russian multiple sclerosis patients. *Pharmacogenomics* 13, 43–53. doi: 10.2217/pgs.11.136

Vidmar, L., Maver, A., Drulović, J., Sepčić, J., Novaković, I., Ristič, S., et al. (2019). Multiple sclerosis patients carry an increased burden of exceedingly rare genetic variants in the inflammasome regulatory genes. *Sci. Rep*. 9:9171. doi: 10.1038/s41598-019-45598-x

Vilariño-Güell, C., Zimprich, A., Martinelli-Boneschi, F., Herculano, B., Wang, Z., Matesanz, F., et al. (2019). Exome sequencing in multiple sclerosis families identifies 12 candidate genes and nominates biological pathways for the genesis of disease. *PLoS Genet*. 15:e1008180. doi: 10.1371/journal.pgen.1008180

Weber, F., Fontaine, B., Cournu-Rebeix, I., Kroner, A., Knop, M., Lutz, S., et al. (2008). IL2RA and IL7RA genes confer susceptibility for multiple sclerosis in two independent European populations. *Genes Immun*. 9, 259–263. doi: 10.1038/gene.2008.14

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REVIEW
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An "Outside-In" and "Inside-Out" Consideration of Complement in the Multiple Sclerosis Brain: Lessons From Development and Neurodegenerative Diseases

B. Paul Morgan[1], *Jennifer L. Gommerman*[2] *and Valeria Ramaglia*[2]*

[1]*UK Dementia Research Institute at Cardiff, Cardiff University, Cardiff, United Kingdom,* [2]*Department of Immunology, University of Toronto, Toronto, ON, Canada*

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***Correspondence:**
Valeria Ramaglia
v.ramaglia@utoronto.ca

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The last 15 years have seen an explosion of new findings on the role of complement, a major arm of the immune system, in the central nervous system (CNS) compartment including contributions to cell migration, elimination of synapse during development, aberrant synapse pruning in neurologic disorders, damage to nerve cells in autoimmune diseases, and traumatic injury. Activation of the complement system in multiple sclerosis (MS) is typically thought to occur as part of a primary (auto)immune response from the periphery (the outside) against CNS antigens (the inside). However, evidence of local complement production from CNS-resident cells, intracellular complement functions, and the more recently discovered role of early complement components in shaping synaptic circuits in the absence of inflammation opens up the possibility that complement-related sequelae may start and finish within the brain itself. In this review, the complement system will be introduced, followed by evidence that implicates complement in shaping the developing, adult, and normal aging CNS as well as its contribution to pathology in neurodegenerative conditions. Discussion of data supporting "outside-in" vs. "inside-out" roles of complement in MS will be presented, concluded by thoughts on potential approaches to therapies targeting specific elements of the complement system.

Keywords: complement, multiple sclerosis, pathology, outside-in, inside-out

INTRODUCTION

Multiple sclerosis (MS) is traditionally considered to be a chronic immune-mediated demyelinating disease of the central nervous system (CNS) mediated by autoreactive lymphocytes that are primed against a CNS antigen in the periphery, enter the CNS *via* breached blood-brain barriers (BBB), and are reactivated in the perivascular spaces of postcapillary venules and meningeal vessels (Engelhardt et al., 2016). As such, one could think of MS as a disease mediated by "outside-in" mechanisms, where the peripheral immune system from the "outside" enters the "inside" of the brain causing injury. Indeed, currently approved disease-modifying treatments (DMT),

which are effective in modulating peripheral immunity, reduce demyelinating lesions in relapsing-remitting MS (RRMS). The "outside-in" hypothesis also finds support in genome-wide association studies that identified immune-related susceptibility gene variants in a large cohort of MS patients (International Multiple Sclerosis Genetics Consortium, 2019).

An alternative theory that argues against a causal role of immune cells in the initiation of MS proposes that a primary "cytodegeneration" in the CNS, originating at oligodendrocytes and/or neurons, starts years before the manifestation of any clinical symptoms. In susceptible individuals, this CNS-intrinsic cytodegeneration is followed by an autoimmune inflammatory reaction against antigens that are shed as a result of the primary cellular damage (Stys et al., 2012b). This alternative hypothesis centered on "inside-out" disease mechanisms, finds support in ultrastructural evidence of earliest myelin changes identified in the layer of the myelin sheath that is nearest the axon (Rodriguez and Scheithauer, 1994), in pathological evidence of myelin and axonal degeneration that is not accompanied by evidence of an adaptive immune response in the normal-appearing white matter (Trapp et al., 1998; Henderson et al., 2009), and in clinical evidence of the inability to stop disease progression using immunomodulatory drugs (reviewed in Ciotti and Cross, 2018).

Overall, while inflammatory processes are detected in the CNS of MS patients both at the early and late disease stages (Frischer et al., 2009; Machado-Santos et al., 2018; Fransen et al., 2020) and are undoubtedly important in shaping pathological processes, the evidence is equally consistent with either primary autoimmune pathogenesis of MS ("outside-in" hypothesis) or a model in which an initial injury causes shedding of a high level of autoantigens, which in turn triggers a secondary inflammatory reaction ("inside-out" hypothesis). Therefore, the question remains: is MS initiated by "outside-in" or "inside out" disease mechanisms?

One piece of the puzzle is the complement system, a major arm of the innate immune system that has been implicated in the pathogenesis of MS since the early 1970s (Lumsden, 1971). While it is widely assumed that, in MS, complement proteins derived from the circulation enter the brain parenchyma through a leaky BBB to tag antigen-antibody complexes ("outside-in" paradigm), a potential role of CNS-derived complement components in MS disease processes that originate within the CNS ("inside-out" paradigm) has been largely ignored. This review focuses on how the complement proteins can shape the MS-affected CNS.

THE COMPLEMENT SYSTEM

Complement plays a central role in the innate immune system. It consists of approximately 50 fluid-phase and cell surface-associated proteins that provide the first line of defense against pathogens and clear immune complexes by tagging and mediating removal of non-self (i.e., pathogens) or altered-self (i.e., dead and dying cells) antigens (Ricklin et al., 2010). Detailed schematics of components and activation products of the complement cascade have been previously published including recently (Morgan and Harris, 2015; Carpanini et al., 2019), and are also shown in **Figure 1**. The complement system is activated *via* three initiating pathways (classical, alternative, and lectin). Regardless of the initiating pathway, complement activation converges on a common effector pathway, terminating in the formation of the terminal complement complex (TCC) that, in the context of cell membranes, creates a transmembrane pore, the membrane attack complex (MAC). The classical pathway is activated by binding of C1q, a component of the C1 complex, to the Fc domain of antibodies bound to antigens or directly to "danger" epitopes. The lectin pathway is initiated by the binding of mannose-binding lectin to certain carbohydrates expressed on the pathogen surface, whereas the alternative pathway is a powerful amplification loop initiated either spontaneously by low-rate hydrolysis of C3 or by C3b generated in the classical/lectin pathways and progresses through binding of activated factor B and generation of a C3-cleaving enzyme (convertase) on surfaces that lack complement inhibitors (Ricklin et al., 2010).

Regardless of the pathway of activation, complement acts through the production of opsonins (C3b, iC3b, C4b, et cetera) which are molecules that enhance the ability of macrophages and neutrophils expressing complement receptors to phagocytose material; anaphylatoxins (C3a, C5a) which are peptides that induce local and systemic inflammatory responses, increasing the permeability of blood vessels and attracting neutrophils through their chemotactic properties; and through direct killing of organisms by the MAC, which disrupts and forms pores in the phospholipid bilayer of a target cell (Ricklin et al., 2010).

While complement components exert their effector functions against "danger" signals on pathogens, healthy self tissue is protected from undesired complement activation by complement inhibitors present either in the fluid-phase or on membrane surfaces (Hajishengallis et al., 2017). Key complement regulators are indicated in **Figure 1**. A tight balance between activation and regulation keeps complement in check, whereas disruption of this balance caused by over-activation and/or insufficient regulation can result in tissue injury (Ricklin and Lambris, 2013).

SOURCES OF COMPLEMENT PROTEINS

Although complement components in plasma are synthesized mainly by hepatocytes in the liver, the discovery that CNS-resident cells such as glial cells and neurons can produce complement proteins (Levi-Strauss and Mallat, 1987; Morgan and Gasque, 1997; Gasque et al., 2000), was a prelude to the changing view of immune privilege in the CNS (Engelhardt and Coisne, 2011). For example, it is now appreciated that specialized pockets in the brain are not immune privileged. In particular, the dura that lies above the leptomeninges is transversed by functional lymphatics and fenestrated vasculature that lacks tight junctions, making it highly permeable to peripheral immune cells and soluble molecules (including complement proteins) that enter into the dura, even at a steady-state (reviewed in Rua and McGavern, 2018; Ramaglia et al., in press b). Therefore, in addition to a parenchymal source of complement, serum-derived

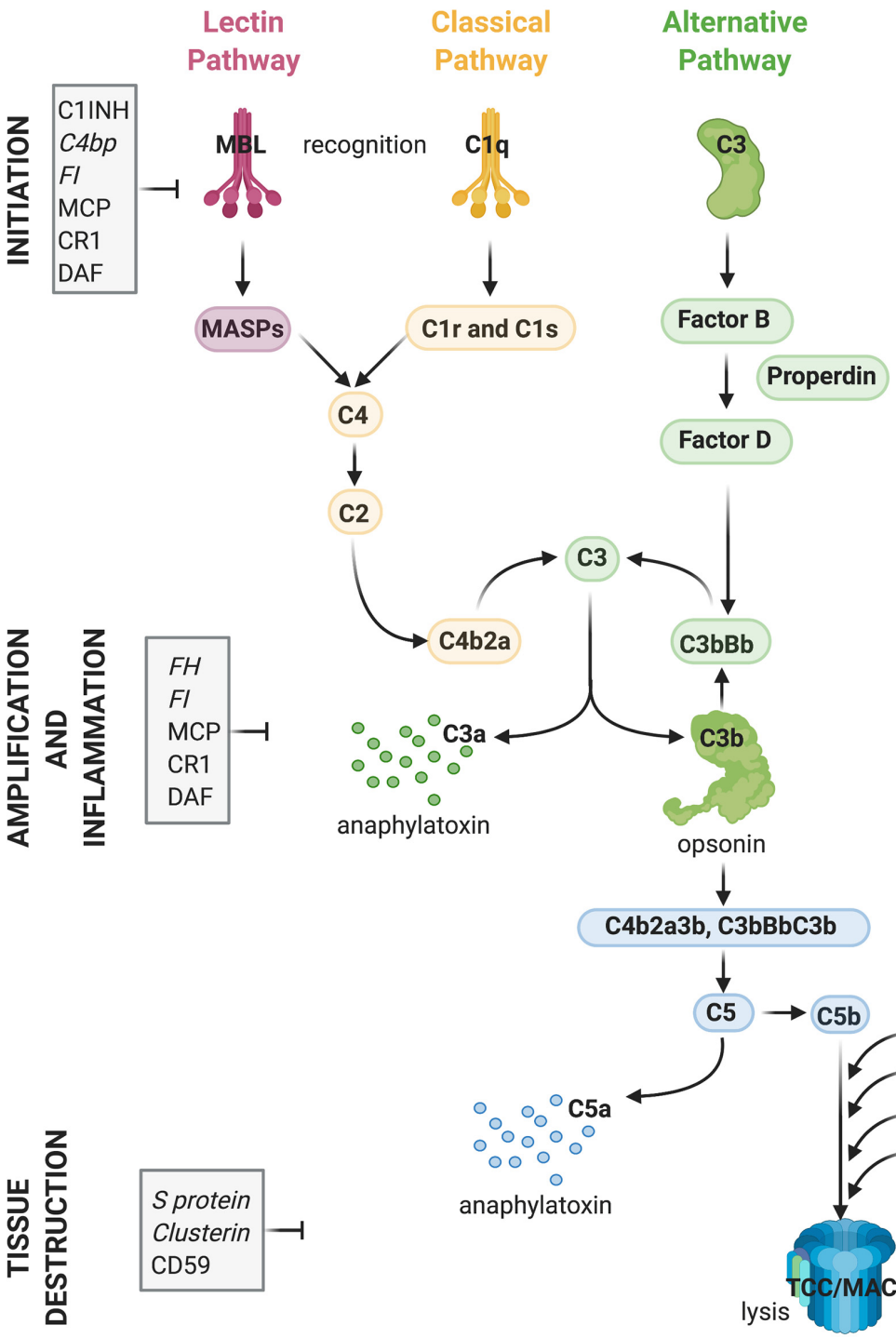

FIGURE 1 | Activation and regulation of the complement (C) system. Recognition of target epitopes by C1q (Classical pathway) or MBL (Lectin pathway) result in the cleavage of C4 and formation of the C3 convertase, cleaving C3. C3 is also activated *via* the alternative pathway by a constant "tick-over" that can be amplified during pathological conditions. Cleaved C3 can nucleate the formation of the C5 convertase, cleaving C5 and eventually forming the terminal complement complex or membrane attack complex (TCC, MAC, C5b9), which can lyse membranes and/or induce cell activation. Activation of C4, C3, and C5 lead also to the formation of anaphylatoxins which cause chemotaxis and inflammation. Activation of the complement system is tightly regulated by soluble (shown in italics) and membrane-bound proteins which can either inhibit the formation or accelerate the decay of the convertases or impede assembly of the C5b9 complex. Abbreviations: C1INH, C1 inhibitor; C4bp, C4 binding protein; FI, factor I; MCP, membrane co-factor protein; CR1, complement receptor 1; DAF, decay-accelerating factor; FH, factor H; MBL, mannose-binding lectin.

complement can enter the brain either through a leaky blood-brain barrier (BBB) and/or through a normally highly permeable meningeal brain barrier.

While little is known about the synthesis of terminal complement components in the brain (or whether this occurs at all), C1q and C3 synthesis in the brain has been studied in more detail. Astrocytes have been shown to produce C3 and Factor B, components of the alternative complement pathway, *in vitro* (Levi-Strauss and Mallat, 1987). Cultured human microglia were also shown to express C1q, C3, and C4 (Walker et al., 1995). Glioma cell lines have also been a useful tool to study complement biosynthesis by glial cells and have shown to be able to produce proteins of the complement alternative pathway, namely C3, factor B, factor H, and factor I (Gasque et al., 1992) and proteins of the classical complement pathway, namely C1q, C1r, C1 s, C1-Inh, C2, C4, and C5 (Gasque et al., 1993). Astrocytes and microglia also express receptors for the complement activation products C3d and C3a (Gasque et al., 1996) and C5a (Gasque et al., 1995, 1997). In addition to glial cells, also neuronal cell lines can express complement components and regulators (i.e., C3, factor H (FH), factor B (FB), C4, C1-inhibitor (C1-inh), C1q, C5, C6, C7, and C9; Thomas et al., 2000). Early studies have also shown local production of complement proteins in the nervous system *in vivo* in response to peripheral nerve injury (Svensson and Aldskogius, 1992) or experimental lesions in the rat brain (Pasinetti et al., 1992).

More recent studies have shown that during the development of the visual system, synthesis of C1q is upregulated in neurons (Stevens et al., 2007). C1q mRNA and protein expression are also dramatically increased during normal aging of the mouse and human brain (Stephan et al., 2013; see "Early Complement Components in the Developing, Adult, and Normal Aging CNS" section). Using *in situ* hybridization, western blot, and immunohistochemical analysis of the myelinated and demyelinated MS hippocampus compared to controls, Michailidou and colleagues showed that C1q is synthesized also by hippocampal neurons and its expression increases intracellularly in neurons within MS tissue (Michailidou et al., 2015). A subsequent study showed that neuronal C1q synthesis, identified by *in situ* hybridization, occurs also in other areas of cortical gray matter in the MS brain (Watkins et al., 2016; see "Evidence Supporting the 'Outside-In' Paradigm" section). However, one study contradicting the concept that neurons represent an important source of C1q showed that microglial-specific deletion of C1q in mice results in lack of C1q in the adult brain while blood C1q levels remain unchanged, implicating microglia as the predominant CNS source of C1q (Fonseca et al., 2017).

In terms of C3, its synthesis has been reported in reactive astrocytes in MS and other neurodegenerative diseases, with C3 now being considered a marker for the designation of "A1" neurotoxic astrocytes (Liddelow et al., 2017). A1 astrocytes lose their ability to promote neuronal survival, outgrowth, and synaptogenesis, and instead induce the death of neurons and oligodendrocytes (Liddelow et al., 2017). Another study has shown neuronal synthesis of C3 in advanced MS cases. These C3-producing neurons were observed close to $C3d^+$ microglial clusters at the edge of slowly expanding lesions (see "Evidence Supporting the 'Inside-Out' Paradigm" section; Michailidou et al., 2017). Altogether these data show that early complement components, particularly C1q and C3, are present in both the developing brain and normal aging brain independent of the breakdown of the blood-brain barrier, while the synthesis of complement proteins by neurons and glia is increased in the diseased brain, including in MS.

EARLY COMPLEMENT COMPONENTS IN THE DEVELOPING, ADULT, AND NORMAL AGING CNS

Stevens et al. (2007) were the first to demonstrate a substantial role for the upstream "early" classical complement pathway (C1q and C3) in eliminating redundant synapses during the development of the retinogeniculate system in the visual thalamus. In this system, extranumerary synapses are targeted by the complement component C1q, opsonized by C3 and phagocytosed by microglia *via* the complement receptor CR3 (Schafer et al., 2012), in a process which appears to be independent of the formation of the terminal "late" activation effector TCC/MAC (**Figure 2A**). This is supported by experimental studies in mice, where deletion of C1q, C3 or CR3, or pharmacological (minocycline) disruption of microglia-mediated engulfment of synapses during early development leads to defects in eye-specific segregation of retinal ganglion cell projections (Stevens et al., 2007; Schafer et al., 2012). Therefore, the classical complement cascade is involved in mechanisms of synaptic refinement, similarly to what has been previously described for other molecules. These include pattern recognition molecules such as pentraxins (Bjartmar et al., 2006), the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2; Filipello et al., 2018), and the Class I Major Histocompatibility Complex. Involvement of the latter has been shown using mice in which deletion of two of the MHCI genes [H2-K(b) and H2-D(b)], impairs developmental refinement of retinogeniculate projections (Corriveau et al., 1998; Datwani et al., 2009).

During normal development, the complement components that participate in the pruning of synapses are likely produced locally in the brain since the BBB protects the CNS parenchyma from plasma-derived complement and immune cells. As mentioned, C1q can be expressed by microglia and certain neurons such as the retinal ganglion cells (RGC) in the visual system (Stevens et al., 2007). The cytokine transforming growth factor-β (TGF-β) has been identified as a factor secreted by astrocytes that is necessary and sufficient for C1q expression in purified RGC (Bialas and Stevens, 2013). *In vivo*, TGF-β receptors (TGFβRII) are expressed in RGC during development and specific disruption of TGFβRII in retinal neurons significantly reduces C1q expression, decreases synaptic localization of C1q, and inhibits synaptic pruning in the visual thalamus (Bialas and Stevens, 2013). Other cytokines also play roles in synapse pruning during development. For example, the interleukin-1 family cytokine interleukin-33 (IL-33) orchestrates astrocyte-microglial communication to

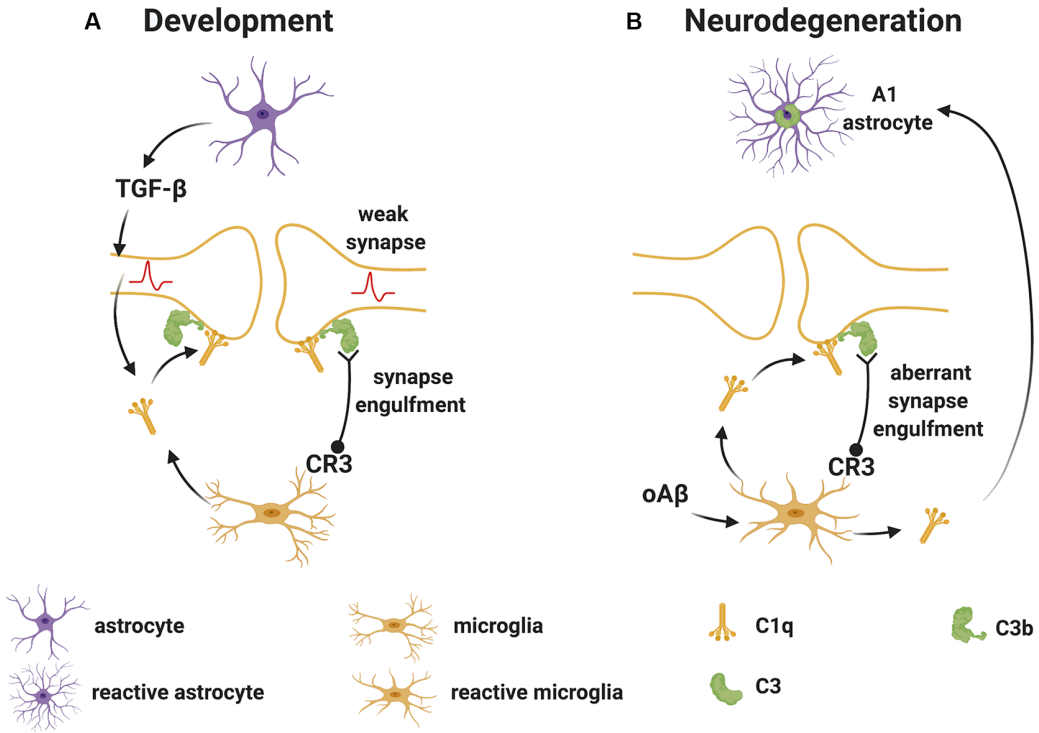

FIGURE 2 | Complement-mediated synapse elimination during development and in neurodegenerative diseases. **(A)** In the developing brain, astrocytes produce TGF-β that promotes the production of C1q by neurons. Neuron-derived and microglia-derived C1q tags weak synapses activating the classical complement pathway which results in the cleavage of C3 with deposition of C3b at synapses. C3b-tagged synapses are then removed through phagocytosis by microglia expressing the complement receptor CR3. **(B)** In neurodegenerative diseases, such as Alzheimer's disease (AD), oAβ promotes the production of C1q by microglia. C1q-tagged synapses are then eliminated by microglia *via* the classical complement pathway as observed for synapses during development. Also, microglia-derived C1q (together with IL-1α and TNF) induces a subtype of $C3^+$ reactive astrocytes, termed A1 astrocytes, which are neurotoxic. Abbreviations: oAβ, oligomeric amyloid β; TGFβ, transforming growth factor β; IL, interleukin; TNF, tumor necrosis factor.

tune microglial engulfment of synapses (Vainchtein et al., 2018). IL-33 is produced by synapse-associated astrocytes and signals to microglia to promote increased synaptic engulfment; deletion of IL-33 leads to excess synapses and abnormal thalamic and sensorimotor (spinal cord) circuit function, demonstrating the requirement for IL-33 in synaptic development in the thalamus and spinal cord (Vainchtein et al., 2018).

The difference between synapses that are tagged for elimination by complement vs. those that are spared is a key question. Certain cell surface markers are implicated as discriminating signals that direct the sparing of synapses. For example, a recent study demonstrated that CD47, the high-affinity receptor for thrombospondin-1, is required to prevent excess pruning of retinogeniculate synapses during development (Lehrman et al., 2018). CD47 is a "don't eat me" signal and self-associated molecular pattern (SAMP) that is expressed on many cell types throughout the body (Reinhold et al., 1995), and is also found on synapses (Mi et al., 2000). It can directly inhibit phagocytosis by binding the signaling molecule SIRPα, on macrophages and other professional phagocytes (Oldenborg et al., 2000; Okazawa et al., 2005). CD47 can also prevent phagocytosis of cells that are opsonized with "eat me" flags, such as complement, showing that it can overrule these signals (Oldenborg et al., 2001). Interestingly, CD47 was shown to preferentially localize at more electrically active synapses in wild-type mice (Lehrman et al., 2018), pointing to the sparing of "stronger" or more active inputs. In line with this finding, CD47 knockout mice did not show the expected preferential microglial engulfment of less active inputs, and both CD47 knockout and SIRPα knockout mice displayed excess microglial engulfment of retinal ganglion cell synaptic inputs (Lehrman et al., 2018). Together, these data suggest that CD47 expression protects certain (potentially more active) synaptic populations from targeting by microglia, while $CD47^{low}$ synapses may be preferentially targeted for removal. Another mechanism potentially regulating the process of C1q-mediated elimination of synapses involves apoptosis. Proteomic investigation and pathway analysis of C1q-tagged synaptosomes revealed the presence of apoptotic molecules (Vdac1, Prdx6, Uchl1, Eno1, Ppp3r1, and Nptx1) in C1q-tagged synapses (Györffy et al., 2018), suggesting that synaptic pruning may involve some of the same molecular triggers that normally stimulate the homeostatic (non-phlogistic) process of complement-mediated enhanced clearance of apoptotic cells in the periphery (Ricklin et al., 2010).

Pruning of excessive synapses does not only occur during postnatal CNS development but is also involved in the dynamic remodeling of synapses which occurs constantly in mature neurons throughout life as a result of experience and learning (Trachtenberg et al., 2002; Tropea et al., 2010; Fu and Zuo, 2011). A recent study showed that the C1q-dependent classical complement pathway is actively involved in synapse elimination by microglia in the healthy adult hippocampus (Wang et al., 2020). C1q was found to localize to synapses and dendritic spines of engram cells, the neurons responsible for the storage of memory in the hippocampus. The identification of C1q-tagged synaptic components within the lysosomes of microglial cells and the finding that depleting microglia or inhibiting microglia-mediated phagocytosis prevented loss of synapses and memory impairment, indicated that microglia are responsible for the elimination of synapses in the healthy brain and that complement provides the relevant phagocytic signal. Also, specific overexpression of the complement regulator CD55 in engram cells protected from memory loss, further indicating that the elimination of synaptic elements by microglia in the healthy adult hippocampus occurs in a complement-dependent manner. In contrast, inhibiting the activity of engram cells exacerbates memory loss, and this could be blocked by depleting microglia or inhibiting complement pathways in engram cells (Wang et al., 2020). These data indicate that synapse elimination by microglia in the adult brain is also activity-dependent, following similar rules to those in the developing brain (Schafer et al., 2012), thus resulting in the erasure of less-active synapses.

Finally, C1q-dependent reorganization of brain circuitry also occurs during normal aging. C1q levels in the brain dramatically increase during aging (by as much as 300-fold in 24 months old mice compared to early postnatal levels and by 8-fold in hippocampi from 75–77-year-old humans compared to 1–2 months old infants) and C1q can be found close to synapses. Importantly, C1q-deficient mice exhibit enhanced synaptic plasticity and less cognitive and memory decline when aged (Stephan et al., 2013).

EARLY COMPLEMENT COMPONENTS IN NEURODEGENERATION

Given that complement plays a key role in developmental synapse elimination, an intriguing hypothesis is that these normal developmental mechanisms may become reactivated during neurodegeneration, driving pathology in settings such as Alzheimer's disease (AD) and Prion disease. Indeed, in AD and prion disease, neuronal death is preceded by synaptic dysfunction and loss (Schneyer and Hall, 1975; Mallucci, 2009). If synaptic alterations indeed precede and predict neuronal death, then early targeting of the pathways responsible for synaptic injury would be an obvious therapeutic approach to prevent loss of neurons. Since the involvement of key complement factors in neurodegeneration has been previously reviewed (Bonifati and Kishore, 2007; Brennan et al., 2016; Carpanini et al., 2019; Lee et al., 2019) including recently in AD (Tenner, 2020), this section will focus on the emerging evidence that supports a role for complement activation in driving loss of synapses early in the disease.

The initial evidence implicating classical complement pathway components in synaptic loss during neurodegeneration comes from studies in glaucoma, a disease characterized by elevation of intraocular pressure, loss of RGC neurons, and degeneration of the optic nerve, eventually resulting in blindness (Williams et al., 2016). Expression profiling approaches in glaucomatous DBA/2J mice that develop pathology closely resembling the human disease showed that the expression of classical complement components is upregulated in the mouse retina during early glaucoma stages, before detectable signs of neurodegeneration (Steele et al., 2006; Howell G. R. et al., 2011). Furthermore, C1q immunoreactivity was upregulated in the layer of the retina enriched in synapses, specifically post-synaptic connections of RGCs, and increased C1q expression was temporally correlated with a decrease in synaptic density (Stevens et al., 2007). Importantly, the most compelling evidence that complement-mediated elimination of synapses may be an early and injurious event in glaucoma is the demonstration that either deletion of C1q in DBA/2J mice or C1 inhibition in a rat glaucoma model confers neuroprotection in the glaucomatous eye (Howell G. R. et al., 2011; Williams et al., 2016).

As mentioned, the complement system has also been implicated in the pathology of the AD brain where activation of virtually all complement components and activation products have been detected (Veerhuis et al., 2011). In terms of early complement proteins, C1q, C3b, C4b, and properdin have all been localized to key pathological hallmarks of AD, such as amyloid plaques and neurofibrillary tangles (hyperphosphorylated τ) in human AD and animal models, supporting the activation of both classical and alternative pathways *in vivo* (reviewed in Fonseca et al., 2011; Veerhuis, 2011). In this context, complement activation may be beneficial through the opsonization and clearance of misfolded proteins (Maier et al., 2008; Fu et al., 2012). Also, C1q has been shown to promote the clearance of apoptotic neurons and neuronal blebs in the AD brain (Fraser et al., 2010). Importantly, sites of protein aggregates and dead cells are decorated with membrane-bound and soluble complement regulators, such as CD55, Factor H, and C4 binding protein (C4BP; Strohmeyer et al., 2002; Trouw et al., 2008; Martin and Blom, 2016), demonstrating an orchestrated mechanism of a complement-mediated process comprising activation that is locally controlled to allow clearance of targeted tissue while limiting activation downstream of the C3 and C5 convertases and the associated pro-inflammatory activities.

Studies in mouse models of AD have helped our understanding of the roles and consequences of early complement activation in AD pathology. For example, human amyloid precursor protein (hAPP) transgenic mice deficient in C3 or overexpressing the C3 convertase inhibitor complement receptor 1-related protein y (Crry), showed greater amyloid accumulation and greater cognitive deficits than control mice, suggesting a protective role for C3 activation (Wyss-Coray et al., 2002; Maier et al., 2008). In contrast, C1q deletion in the

Tg2576-transgenic mouse model of AD resulted in diminished plaque burden, a reduced loss of hippocampal synapses, and less cognitive decline relative to C1q sufficient Tg2576 mice, suggesting a detrimental role for C1q in the model (Fonseca et al., 2004). Also, microglia-derived C1q (together with IL-1α and TNF) induced astrocytes to adopt a neurotoxic A1 phenotype (Liddelow et al., 2017). In more recent studies in other AD models, C1q was found to be increased and deposited at synapses before overt plaque deposition, whereas deletion of C1q or C3 or the complement receptor CR3 (also expressed on microglia), reduced the number of phagocytic microglia as well as the extent of early synapse loss, resulting in improved cognitive function (Hong et al., 2016; **Figure 2B**). Similar evidence of the involvement of early complement components in pathological synapse elimination has been reported in models of frontal temporal dementia (Lui et al., 2016) and West Nile virus infection (Vasek et al., 2016).

Loss of synapses and upregulation of complement components are also major events in other neurodegenerative diseases including Huntington's disease (Moller, 2010) and Parkinson's disease (Chao et al., 2014), however, it is unclear whether in these conditions complement plays a crucial role in the elimination of synapses.

A LINK BETWEEN INFLAMMATION AND INJURY IN THE MS-AFFECTED CNS

The past decade has produced a wealth of studies aimed at understanding the link between inflammation and CNS injury in MS, especially in the later disease stages. Most patients present with RRMS, but the disease eventually transitions into a progressive form (secondary progressive multiple sclerosis, SPMS), and a minority of patients develop progressive disease from the onset (primary progressive multiple sclerosis, PPMS; Lublin et al., 2014). While DMT have been successful in controlling the RRMS form of the disease, they, unfortunately, perform poorly in preventing disease progression and accumulation of clinical disability in PPMS and SPMS patients (Macaron and Ontaneda, 2019). If on one hand, these clinical observations have validated a key role of inflammation from the "outside" in the pathogenesis of RRMS, on the other hand, they have raised doubts regarding whether similar mechanisms are at play during the progressive stage of the disease. The reason DMT fail in SPMS and PPMS may be that the inflammatory targets of these DMT are not relevant in progressive forms of MS. Alternatively, inflammation may still be involved during the progressive phase of the disease, but such inflammatory processes become increasingly compartmentalized within the CNS behind a relatively intact blood-brain barrier (Kutzelnigg and Lassmann, 2014). This hypothesis is supported by pathological (Serafini et al., 2004; Lassmann et al., 2007; Magliozzi et al., 2007, 2010; Howell O. W. et al., 2011; Howell et al., 2015; Lucchinetti et al., 2011; Popescu et al., 2011; Choi et al., 2012; Haider et al., 2016) and imaging studies (Absinta et al., 2015; Zurawski et al., 2020) which—except for some studies (Kooi et al., 2009; Ighani et al., 2020)—have identified aggregates of immune cells in the meninges of MS patients overlaying areas of cortical injury. While these studies implicate inflammatory events within the CNS compartment, this theory still puts the immune system upstream of the disease mechanisms in MS ("outside-in" hypothesis; Kutzelnigg and Lassmann, 2014). Overall, it is now widely accepted that inflammation persists and is linked to tissue injury in both white and gray matter tissue of patients with progressive disease (Magliozzi et al., 2007, 2010; Frischer et al., 2009; Howell O. W. et al., 2011; Machado-Santos et al., 2018); however, the evidence is equally consistent with either the "outside-in" or the "inside-out" paradigm (Stys et al., 2012b). In this section, we present the evidence linking key features of progressive MS white and gray matter pathology with inflammation and specifically complement activation.

MS White Matter

Early pathological studies had already demonstrated that focal demyelinated lesions in the white matter are generally centered on large or medium-sized veins (Rindfleisch, 1863), pointing to a link between events in the periphery and white matter injury. Follow up studies have further highlighted the relationship between inflammation and white matter pathology by showing that lymphocytes and monocytes/macrophages accumulate in the perivascular spaces of medium-sized and small veins within MS plaques. These inflammatory cells can also infiltrate the tissue parenchyma, while local microglial cells become activated (Charcot, 1880). In addition to the lesions, unlesioned areas of the MS-affected CNS also undergo changes that prelude further injury. The so-called "normal-appearing white matter" is characterized by perivascular inflammatory infiltrates, astrocytic gliosis, diffuse axonal injury, and diffuse microglial activation that increases with longer disease duration (Kutzelnigg et al., 2005)—thus, not so normal. Also, within unlesioned tissue, aggregates of HLA^+ microglia/macrophages are observed in proximity to microvessels surrounded by lymphocytic infiltrates identifying "(p)reactive lesions" (**Figure 3A**), so-called since they may or may not represent the earliest stages in the formation of plaques (Barnett and Prineas, 2004; Marik et al., 2007). Active lesions are highly inflammatory and are found most frequently in RRMS (**Figure 3B**). They are characterized by loss of myelin, with HLA^+ and $CD68^+$ microglia/macrophages infiltrating across the lesion. Foamy microglia/macrophages within an MS lesion indicate ongoing phagocytic activity and myelin proteins are detected within the cytoplasm of these myeloid cells (Lucchinetti et al., 2000; Kuhlmann et al., 2017). Histological studies using newly identified markers selectively expressed on microglia, including TMEM119 which differentiates yolk sac-derived CNS-resident microglia from bone marrow-derived recruited monocytes/macrophages, and the purinergic receptor P2RY12 which identifies a homeostatic microglia phenotype (Butovsky et al., 2014; Masuda et al., 2020), showed that ∼45% of macrophage-like cells are derived from the resident microglia pool in active lesions (Zrzavy et al., 2017). In these lesions, microglia show reduced expression of the homeostatic marker P2RY12, which is reacquired in inactive lesions. T cells and $CD20^+$ B cells are also detected within

active lesions (Machado-Santos et al., 2018). While B cells in the lesion are mostly seen in the perivascular space of the central vein, T cells infiltrate the parenchyma in addition to populating the perivascular space. Some $CD8^+$ T cells feature a more tissue-resident memory phenotype (i.e., $CD8\alpha/\alpha$, $CD103^+$; Machado-Santos et al., 2018). Tissue-resident memory T cells lose expression of surface molecules that are involved in the egress of leukocytes from inflamed tissue (S1P1 or CCR7). These molecular changes have been suggested as a potential mechanism responsible for the compartmentalized inflammatory response in MS lesions (Machado-Santos et al., 2018). In addition to $CD8^+$ T cells, $CD4^+$ T cells are also found in MS lesions and have been shown to produce cytokines such as IL-17 and IL-22 which possibly bind to BBB endothelial cells expressing IL-17 and IL-22 receptors, thereby gaining access to the CNS parenchyma (Kebir et al., 2007). Plasma cells are also detected within MS lesions but their phenotype is less well understood (Machado-Santos et al., 2018). Over time, active lesions develop into mixed active/inactive lesions (**Figure 3C**). Indeed, these types of lesions are most commonly found in patients with advanced (disease duration of more than 10 years) or SPMS disease course. They are characterized by a border of activated HLA^+ and $CD68^+$ microglia/macrophages and a hypocellular core. In a subset of these lesions, called slowly expanding or "smoldering", microglia/macrophages at the rim contain MBP or PLP myelin degradation products in their cytoplasm, indicating either a new wave of inflammation and demyelination, or documenting the last remnant of an earlier demyelinating lesion (Frischer et al., 2015). With time, active and mixed active/inactive lesions transition into inactive lesions (**Figure 3D**). These are indeed the dominant plaque type in patients with advanced disease (duration of more than 15 years) or SPMS without attacks (Frischer et al., 2015). Inactive lesions are demyelinated but hypocellular throughout (Frischer et al., 2015).

Overall the pathology of the white matter shows that inflammation is very much linked to demyelination in the MS brain, but it does not answer whether inflammation is the primary cause of the injury (consistent with the "outside-in" paradigm) or a secondary reaction (consistent with the "inside-out" paradigm).

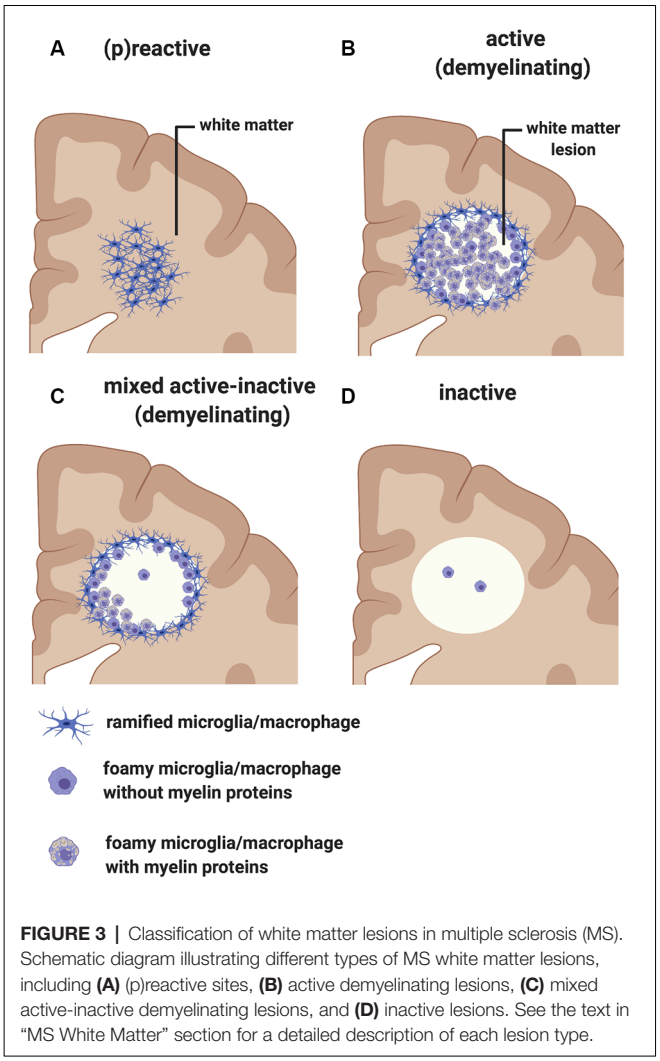

FIGURE 3 | Classification of white matter lesions in multiple sclerosis (MS). Schematic diagram illustrating different types of MS white matter lesions, including **(A)** (p)reactive sites, **(B)** active demyelinating lesions, **(C)** mixed active-inactive demyelinating lesions, and **(D)** inactive lesions. See the text in "MS White Matter" section for a detailed description of each lesion type.

MS Gray Matter

Although gray matter changes in the MS brain were noted early on (Dawson, 1916; Brownell and Hughes, 1962), it was not until the advent of more sensitive imaging and histology techniques that the true extent of gray matter demyelination was appreciated, reaching up to 90% of the cortex in extreme cases (Kidd et al., 1999; Peterson et al., 2001; Bø et al., 2003; Chard and Miller, 2009; Calabrese et al., 2010). Based on the location of the demyelinated area, different types of gray matter lesions have been identified (Bø et al., 2003; Kuhlmann et al., 2017). Subpial lesions, occurring on the surface of the brain and often affecting several adjacent gyri are perhaps the most intriguing since they are unique to MS and not seen in any other neuroinflammatory diseases (Fischer et al., 2013). Therefore, they could hold the key to MS-specific disease mechanisms. Subpial cortical demyelination, although present in earlier stages of MS (Lucchinetti et al., 2011; Popescu et al., 2011), appears to be more dominant in the later progressive stage of the disease (Peterson et al., 2001; Bø et al., 2003; Kutzelnigg et al., 2005). Importantly, subpial changes are not limited to demyelination but also include neuronal (Magliozzi et al., 2010), axonal (Magliozzi et al., 2007), and synaptic (Wegner et al., 2006; Dutta et al., 2011) injury with the extent of subpial cortical damage now considered to be a major contributor to disease progression in MS including both physical (Harrison et al., 2015) and cognitive (Calabrese et al., 2012) impairments. For example, the hippocampus, which is the portion of the cortex critically important for cognitive functions such as the formation, consolidation, and recollection of memories (Squire et al., 2004), is profoundly affected in MS. Not only is the hippocampus extensively demyelinated (Geurts et al., 2007), but it also shows evidence of neuronal injury (Papadopoulos et al., 2009) and synaptic abnormalities (Dutta et al., 2011). Structural and functional disconnections of the hippocampus from several brain networks have also been revealed by magnetic resonance imaging (MRI) studies (Sicotte

et al., 2008; Gold et al., 2010, 2014; Longoni et al., 2015). Experimental (Trapp et al., 2007; Werneburg et al., 2020) and post-mortem (Peterson et al., 2001; Werneburg et al., 2020) studies indicate that disconnection of brain networks in MS may be mediated by the loss of synapses *via* the pruning process, part of which is complement-mediated (see "Evidence Supporting the 'Inside-Out' Paradigm" section).

Whether inflammation causes subpial cortical gray matter damage has been debated. When compared with the highly inflammatory features of white matter lesions described above, subpial gray matter lesions are relatively "non-inflammatory" in nature. They lack evidence of major BBB disturbances (van Horssen et al., 2007) and display a paucity of parenchymal immune cell infiltration (Peterson et al., 2001; Bø et al., 2003). Also, current anti-inflammatory treatments that effectively modulate peripheral immunity do not prevent or resolve gray matter damage (Ciotti and Cross, 2018).

One theory supporting a key role of inflammation in subpial cortical pathology, while reconciling pathological and clinical findings of progressive MS, proposes that this injury may be driven by a compartmentalized immune response involving the inflamed leptomeninges within brains that have a relatively intact BBB (Serafini et al., 2004; Lassmann et al., 2007; Magliozzi et al., 2007, 2010; Howell O. W. et al., 2011; Lucchinetti et al., 2011; Popescu et al., 2011; Choi et al., 2012; Absinta et al., 2015; Howell et al., 2015; Haider et al., 2016). Histological examination of autopsy CNS tissue from progressive MS cases have shown evidence of Tertiary Lymphoid Tissues (TLT) in the leptomeninges lining the forebrain (Serafini et al., 2004; Magliozzi et al., 2007), the cerebellum (Howell et al., 2015) and to a lesser extent the spinal cord (Reali et al., 2020). MRI studies have also confirmed the presence of meningeal TLT in the MS brain (Absinta et al., 2015). Importantly, these leptomeningeal TLT are associated with underlying (subpial pattern) demyelination, neuronal loss, and a gradient of microglial activation from the subpial area moving inward into the tissue (Magliozzi et al., 2010; Howell O. W. et al., 2011). The meningeal TLTs harbor B cells, T cells, dendritic cells, macrophages, monocytes, plasma cells, and stromal cells that resemble Follicular Dendritic Cells (FDC) normally found in germinal centers (Serafini et al., 2004; Magliozzi et al., 2007; Howell O. W. et al., 2011; Lucchinetti et al., 2011; Lagumersindez-Denis et al., 2017; Reali et al., 2020). While germinal center environments can support the secondary diversification of B cell receptors (affinity maturation and class switch) in the context of some animal models of MS (Galicia et al., 2013), a study using post-mortem samples suggested that the majority of these secondary diversification events are occurring within the draining cervical lymph node rather than in the CNS itself (Stern et al., 2014). Nonetheless, it has been shown that within TLT, B cells proliferate and $CD21^+$ $CD35^+$ FDC-like stromal cells produce the chemokine CXCL13, suggestive of an immunocompetent stromal cell niche that serves to attract and retain leukocytes, supporting their survival and proliferation (Serafini et al., 2004).

An important function of cells that reside within the meninges is the ability to secrete by-products which are released into the CSF and may diffuse freely throughout the subarachnoid space. If one considers that there are regions of reduced CSF flow, particularly within brain sulci, such by-products may become concentrated in "hot spots," resulting in the trapping of immune cells (Howell O. W. et al., 2011). These soluble molecules are potentially inflammatory, cytotoxic, myelinotoxic but also potentially anti-inflammatory and neuroprotective. Indeed, recent studies analyzing the content of CSF in MS patients have shown that high cortical lesion load correlates with proinflammatory cytokines (IFNγ, TNF, IL2, and IL22; Gardner et al., 2013; Magliozzi et al., 2018), molecules related to sustained B-cell activity and lymphoid-neogenesis (CXCL13, CXCL10, LTα, IL6; Magliozzi et al., 2018), B-cell survival factors (BAFF; Magliozzi et al., 2019), factors indicative of BBB leakage (fibrin, complement and coagulation factors; Magliozzi et al., 2019) and iron-related proteins (free-hemoglobin and haptoglobin; Magliozzi et al., 2019), but also anti-inflammatory mediators such as IL10 (Magliozzi et al., 2019). In line with the latter, regulatory T (Treg) cells producing IL-10 have been detected in the MS CSF (Feger et al., 2007) although deficits of their regulatory functions and migratory properties have also been reported in MS (Viglietta et al., 2004; Astier et al., 2006; Martinez-Forero et al., 2008; Venken et al., 2008; Schneider-Hohendorf et al., 2010).

In summary, while the spatial association between TLT and subpial injury has suggested a potential role in initiating and/or modulating demyelinating pathology in the gray matter, these same pathological features are equally consistent with a potential reaction of the immune system in the meningeal compartment to areas of pre-existing injury in the underlying cortex. Without longitudinal studies, it is impossible to conclude whether these TLT sites preceed the formation of subpial lesions, or whether they represent an attempt of the immune system to repair an existing injury. If the latter scenario is true, then the presence of meningeal TLT reacting to a site of primary injury would be consistent with the "inside-out" paradigm. Studies in animal models that replicate meningeal TLTs and subpial pathology (Pikor et al., 2015; Ward et al., 2020), emerging MRI approaches to identify and perhaps monitor the formation of TLT in patients (Absinta et al., 2015), and multi-dimensional technologies to phenotype cells *in situ* (Ramaglia et al., 2019) will be important to unravel the nature of the relationship between TLTs and subpial injury in MS.

COMPLEMENT IN MS

A contribution of the complement system to the pathology of MS has long been suspected based on pathological and serological studies in MS as well as functional studies in EAE. However, the extent and trigger(s) of complement activation and the pathways involved in specific disease processes remain unclear. In particular, the relative contribution of serum-derived complement vs. CNS-derived complement in initiating as well as propagating MS disease processes remains to be determined. In this section, the evidence is reviewed that supports a role for complement in both "outside-in" and "inside-out" pathological mechanisms of the MS-affected CNS.

Evidence Supporting the "Outside-In" Paradigm

While the specific antigens responsible for initiating the immune response in MS remain unidentified, MS patients commonly have oligoclonal immunoglobulin G in the CSF (Joseph et al., 2009) and several studies have demonstrated antibodies to myelin and other CNS autoantigens in patients (reviewed in Berger and Reindl, 2007). Moreover, T cells specific for myelin antigens have been detected in the blood of MS patients (reviewed in Kaskow and Baecher-Allan, 2018) and found to produce inflammatory mediators such as granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), interferon-gamma (IFN-γ), interleukin-2 (IL-2) and C-X-C chemokine receptor type 4 (CXCR4; Galli et al., 2019). Importantly, transient gadolinium-enhancing lesions on magnetic resonance scans have indicated BBB breakdown, especially in RRMS patients (Stone et al., 1995). In line with this observation, immunoglobulins and complement deposits have been described in MS lesions (Compston et al., 1989; Storch et al., 1998; Lucchinetti et al., 2000; Prineas et al., 2001; Ingram et al., 2014; Michailidou et al., 2015, 2017; Watkins et al., 2016; Loveless et al., 2018). Also, plasma proteins including Factor XIIa, plasmin, and thrombin, which are part of the coagulation pathway, could leak from the circulation into the brain parenchyma through a compromised BBB, particularly in RRMS patients, triggering direct activation of C3 (Markiewski et al., 2007; Amara et al., 2010) and C5 (Huber-Lang et al., 2006). Notably, *in situ* evidence of antibody and complement deposits within MS plaques in close association with capillary endothelial cells (Compston et al., 1989; Storch et al., 1998),

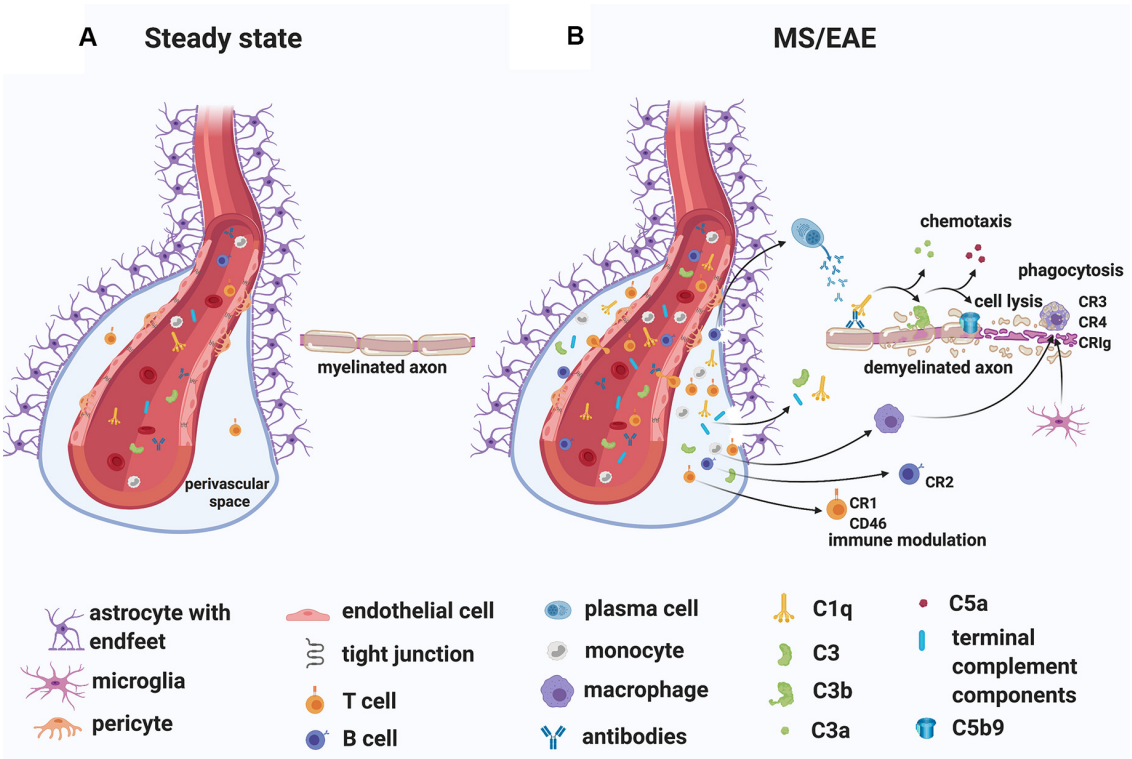

FIGURE 4 | A role for complement in the "outside-in" paradigm of white matter demyelination in MS. **(A)** During steady state, complement components circulate in the blood. Only leukocyte extravasation across the blood-brain barrier (BBB) occurs but is limited to few activated $CD4^+$ T lymphocytes that perform immunological surveillance. These activated lymphocytes interact with adhesion molecules (ICAM-1, VCAM-1) expressed on the lumen of vascular endothelial cells. Chemokine (CXCL12) expression by endothelial cells on the abluminal side contributes to sequestering the activated $CD4^+$ T cells in the perivascular space through binding to the chemokine receptor (CXCR4) on the T cell. **(B)** During MS/EAE, the efficiency of leukocyte diapedesis is increased. CCL19, CCL21, and CXCL12 are up-regulated by cerebrovascular endothelial cells that promote the recruitment and adhesion of $CD4^+$ T cells primed in the periphery against a CNS antigen. On the abluminal surface of endothelial cells, CXCR7 binds to CXCL12 to reduce T cell sequestration in the perivascular space. Within the perivascular space, activated T cells produce chemokines and cytokines (such as TNFα and GM-CSF) that promote the recruitment of myeloid cells from the blood. Matrix metalloproteinases (MMP-2 and MMP-9) are produced and selectively cleave dystroglycan in the astrocytic foot processes, allowing penetration of T cells, B cells, monocytes, and complement into the CNS parenchyma. Within the CNS, encephalitogenic T cells re-encounter their specific antigen and are re-activated, producing inflammatory cytokines. T cells can also bind directly to myelin epitopes producing cytotoxic mediators and leading to activation of macrophages. Infiltrating B cells transition into antibody-producing plasma cells perhaps *in situ*. Autoantibodies can also enter the CNS through a breached BBB. Complement components enter the CNS *via* a breached BBB and are activated either by the recognition of antigen/antibody complexes or directly by "altered self" myelin epitopes. Complement-tagged myelin is then phagocytosed by phagocytes expressing complement receptors (macrophages and microglia). Activated complement components can also bind T cells and B cells *via* their complement receptor modulating their function (see details in text). Abbreviations: CNS, central nervous system; TNFα, tumor necrosis factor α; GM-CSF, granulocyte-macrophage colony-stimulating factor; CR, complement receptor.

have suggested a role for an "outside-in" complement/antibody-mediated injury in lesions (**Figure 4**). Moreover, complement components and regulators are elevated systemically and in the CSF of MS patients (Ingram et al., 2009, 2010a,b, 2012; Zelek et al., 2019) and early complement pathway gene variants (C3, C1QA, and CR1) have recently been associated with structural and functional measures of MS severity (Fitzgerald et al., 2019), indicating an inherent susceptibility to complement-mediated injury in some patients.

A detailed pathological analysis performed by Lucchinetti et al. (2000) on active and early demyelinating MS white matter lesions from a cohort of patients with relatively short disease duration (the mean disease duration before autopsy or biopsy was 39 and 9 months, respectively), identified a histological pattern (pattern II) of lesions with prominent complement activation in a distinct but large subgroup (pattern I: 4%, pattern II: 59%, pattern III: 26%, pattern IV: 11% of total autopsy cases analyzed) of MS patients. In these lesions, the terminal complement TCC/MAC was associated with phagocytic macrophages. These findings suggested that complement activation may be one of at least four possible mechanisms of tissue injury leading to demyelination. Other patterns involve activated macrophages/microglia (but not complement activation; pattern I), apoptotic (pattern III), or non-apoptotic (pattern IV) oligodendrocyte degeneration (Lucchinetti et al., 2000). While this study highlighted the heterogeneity of early MS lesions, a subsequent report by Breij et al. (2008) based on the analysis of white matter lesions in autopsy tissue from MS patients with long disease duration (mean disease duration was 22 years), showed that complement deposits can be found consistently in areas of demyelination across all patients with established MS lesions. The authors suggested that the previously reported heterogeneity of white matter lesions (Lucchinetti et al., 2000) may have been specific to early disease and was therefore not seen in their autopsy samples. Subsequent immunohistochemical studies analyzing the presence and localization of key complement components (C3, factor B, C1q), activation products (C3b, iC3b, C4d, TCC/MAC), regulators (factor H, C1-inhibitor, clusterin) and receptors (C3aR, C5aR1) in established MS lesions found that, although variable between individuals, the presence of complement proteins and activation products in and around white matter lesions is a consistent feature of progressive MS (Ingram et al., 2014; Loveless et al., 2018), echoing the conclusions from Breij et al. (2008).

Evidence for complement activation in MS gray matter has also been mixed. Initial histological studies reported evidence of activated complement (C4d) on oligodendrocytes at the edge of small cortical plaques (Schwab and McGeer, 2002). Subsequent studies in autopsy tissue from MS patients with the long-standing disease showed little evidence for complement activation products in purely cortical lesions (Brink et al., 2005). More recent studies in lesions from progressive MS patients showed evidence of C1q deposition on neurons, as well as complement activation fragments (Bb, C3b) on neurons and glia, and TCC/MAC on neurons across cases analyzed (Watkins et al., 2016).

Intriguingly, complement may also play a role in the context of compartmentalized inflammation within meningeal TLT (see "MS Gray Matter" section; Ramaglia et al., in press a). Using the Th17 cell A/T model to induce EAE in SJL/J mice, Pikor et al. (2015) demonstrated that at the earliest stages of CNS autoimmunity, meningeal TLTs contain cells that are positive for the complement component C3. Although the nature of the $C3^+$ cells within TLTs was not explored, deposited C3 could modulate adaptive immune cell responses. For example, activated C3 (C3d) bound to antigens has been shown to regulate B cell function by lowering the threshold for B cells activation through its interaction with the CR2 co-receptor on B cells (Dempsey et al., 1996). In terms of T cells, intracellular expression and activation of C3 have been implicated in the control of human adaptive T cell responses (Liszewski et al., 2013). One hypothesis is that C3a-like and C3b-like products are continuously generated intracellularly within endosomal/lysosomal compartments at low levels in resting T cells *via* cathepsin-L (CTSL)-mediated cleavage of C3; the C3a-like product engages the lysosomal-expressed G-protein-coupled C3a receptor (C3aR) to sustain the tonic activity of mammalian target of rapamycin complex 1 (mTORC1) and survival of circulating T cells at steady-state (Liszewski et al., 2013). The CTSL-mediated activation of C3 is increased by T cell receptor (TCR) activation and CD28 co-stimulation. C3 activation fragments then shuttle to the cell surface where they engage their respective receptors, C3aR (which binds C3a) and CD46 (which binds C3b), to induce IFN-γ production and T helper type 1 (Th1) differentiation (Liszewski et al., 2013). The same pathway could function in human $CD8^+$ T lymphocytes. According to this hypothesis, C3 protein is processed intracellularly into activation fragments that engage the same receptors in an autocrine/paracrine manner and drives IFN-γ production and cytolytic activity (Arbore et al., 2018).

In line with the potential production and secretion of complement proteins by immune cells residing in meningeal TLTs, a recent proteomic CSF profiling in early MS patients showed that among 227 proteins differentially expressed between the patients with high vs. low cortical lesion load, 30% were related to the complement cascade, suggesting, that in addition to other soluble mediators, complement positively correlates with cortical damage at early disease stages (Magliozzi et al., 2019), possibly by diffusing through a disrupted glial limitans into the subpial cortex causing injury. Other proteins identified in the CSF of MS patients included the blood coagulation factor fibrinogen (Magliozzi et al., 2019), suggesting that the extrinsic pathway of the coagulation system may also be involved in pathological processes occurring in the MS brain. From the earliest studies using experimental animal models of demyelination, particularly EAE, it was already noted that perivascular deposition of insoluble fibrin [produced from fibrinogen by perivascular tissue factor and procoagulant proteins (Thomas et al., 1993; Akassoglou and Strickland, 2002)], occurred in conjunction with paralytic episodes (Paterson, 1976). Further studies causing abnormal cleavage and degradation of fibrinogen (Adams et al., 2004) or blocking the conversion of fibrinogen into insoluble fibrin (Akassoglou et al., 2004; Yang et al., 2011), reduced EAE severity.

Experimental studies into the mechanisms of fibrin-induced injury performed by genetically blocking the ability of fibrin to bind the CD11b/CD18 integrin receptor on microglia and macrophages (without affecting the binding of other ligands to CD11b/CD18), resulted in reduced microglial activation, decreased axonal damage, less demyelination, and reduced paralysis, demonstrating that fibrinogen entry into the CNS and subsequent CD11b-mediated microglial activation may be key upstream molecular events that drive inflammatory demyelination (Adams et al., 2007; Davalos et al., 2012). More recently, it was shown that fibrinogen stored in extracellular vesicles from blood plasma induces encephalitogenic $CD8^+$ T cells, contributing to the perpetuation of neuroinflammation and relapses in response to immunization with a myelin antigen ($MOG_{35–55}$; Willis et al., 2019).

Indications that complement plays a role in the pathology of MS comes from experimental studies in animal models of demyelination, particularly EAE. From initial studies that have used cobra venom factor as a tool to consume complement, to subsequent studies that have used either untargeted or targeted pharmacological inhibition of complement activation or that have used genetic deletion of complement components and regulators, it has become apparent that reducing complement activation in EAE is protective (reviewed in Ingram et al., 2009). In particular, the "outside-in" role for systemically-derived complement in models of demyelination comes from recent evidence in the chronic relapsing EAE (crEAE) model of demyelination in Biozzi AB/H mice (Michailidou et al., 2018). In these mice, immunization with homogenates of the syngeneic spinal cord (SCH) in complete Freund's adjuvant induces a chronic relapsing form of EAE (Baker et al., 1990), which pathologically is characterized by inflammatory demyelinating lesions in the spinal cord and substantial axonal injury (Jackson et al., 2009). In terms of inflammation, these spinal cord lesions are populated by macrophages, $CD4^+$ T lymphocytes (Butter et al., 1991), and deposits of TCC/MAC (Ramaglia et al., 2015). Notably, post-symptomatic treatment of crEAE with an antisense oligonucleotide that specifically targets CNS-extrinsic production of murine C6 mRNA (a component necessary for the formation of the TCC/MAC complex), inhibited TCC/MAC deposition inside the CNS, prevented relapses and protected from relapse-induced axonal and synaptic damage (Michailidou et al., 2018). Further analysis showed that this protection was achieved by impeding the activation of parenchymal neuroinflammatory responses, including the Nod-like receptor protein 3 (NLRP3) inflammasome (Michailidou et al., 2018). Therefore antisense-mediated knockdown of C6 expression outside the CNS is sufficient to impede neuroinflammation in the crEAE model of MS, pointing to a pathogenic role of the serum-derived terminal complement components in this model of demyelination.

Evidence Supporting the "Inside-Out" Paradigm

Evidence that complement may be triggered as a reaction to primary damage within the CNS derives from studies in models of traumatic nerve or CNS injury. In these models, the primary injury is a mechanical insult either delivered by a compression of the sciatic nerve, inducing Wallerian degeneration (Ramaglia et al., 2007) or by a weight drop on a closed skull in the case of a traumatic brain injury (TBI; Fluiter et al., 2014) or on the spinal cord in the case of a spinal cord injury (SCI; Qiao et al., 2010). In each instance, the mechanical injury results in the activation of the complement system to completion including the formation of TCC/MAC (Anderson et al., 2004; Ramaglia et al., 2007; Nguyen et al., 2008; Fluiter et al., 2014). Importantly, inhibition of the complement system at various levels of the cascade results in reduced pathology and ameliorated clinical outcome in rodent models of nerve/CNS injury. For example, broad inhibition of the complement system by targeting the C3 convertase reduces neutrophil accumulation (Kaczorowski et al., 1995), promotes neuronal survival, induces neuroprotective intracerebral gene expression, and ameliorates neurological outcome after TBI (Leinhase et al., 2006). More specific inhibition of the complement cascade targeting only the alternative pathway of complement also led to substantial attenuation of cerebral tissue damage and neuronal cell death, inducing a neuroprotective pattern of intracerebral gene expression following TBI (Leinhase et al., 2007). Alternative strategies that have targeted specific pro-inflammatory arms of the complement system, such as inhibiting the anaphylatoxin C5a, have also shown protective effects by reducing neutrophil extravasation into the brain parenchyma after TBI (Sewell et al., 2004). Inhibition of the most downstream component of the terminal complement activation pathway, the formation of the membrane attack complex (TCC/MAC), is also sufficient to prevent secondary neurologic damage and neurologic deficit after TBI (Fluiter et al., 2014; Ruseva et al., 2015). In the case of SCI, deletion of C1q resulted in greater locomotor recovery and better histological outcome compared to wild-type mice after a contusion injury of the spinal cord (Galvan et al., 2008), suggesting that initiation of the classical complement pathway *via* C1q is detrimental to recovery after SCI. Involvement of the classical pathway of complement in determining pathology after SCI was also confirmed by the protective effects of C1 esterase inhibitor (C1-INH) on pathology and function after traumatic SCI in the rat (Tei et al., 2008). Blocking activation of the complement system at its core by deleting C3 or *via* targeted inhibition of C3 activation to sites of C3 deposition (with CR2-Crry) significantly reduced necrosis, demyelination, and neutrophil infiltration, improving functional outcome after SCI in mice (Qiao et al., 2006). Also, deletion of the alternative pathway component Factor B or pharmacological inhibition of the alternative pathway with a monoclonal antibody against Factor B reduced tissue damage and demyelination, reduced inflammatory cell infiltrate, and improved functional recovery after SCI in mice (Qiao et al., 2010). On the contrary, deletion of the TCC/MAC complement regulator CD59a resulted in significantly increased tissue injury and impaired functional recovery compared to wild-type mice (Qiao et al., 2010), also implicating the terminal complement pathway in the nerve injury caused by spinal cord trauma. Likewise in the peripheral nerve, blocking the C3 convertase (Ramaglia et al., 2008) or the TCC/MAC (Ramaglia et al., 2007) reduces pathology and

neurological symptoms after trauma, whereas deletion of CD59a exacerbates Wallerian degeneration (Ramaglia et al., 2009a). Interestingly in the peripheral nerve, inhibition of TCC/MAC also accelerates the regeneration of the nerve (Ramaglia et al., 2009b), further proving a detrimental role of complement activation during nerve damage. Therefore, complement can be activated by degenerating axons in the absence of antibodies, demonstrating a reaction to altered-self epitopes. In the context of MS, a primary injury to myelinated axons or axons undergoing Wallerian degeneration could trigger secondary complement activation (**Figure 5A**).

Additional evidence that CNS-intrinsic complement activation may occur independently of immune cell-mediated demyelination comes from observations in the normal-appearing white matter or periplaque of MS brains. These regions are often reported to contain clusters of microglia (also called microglial nodules) around damaged axons coated with complement deposits (C3d; Prineas et al., 2001; Barnett et al., 2009; Ramaglia et al., 2012). While these C3d-associated microglial nodules have been proposed to play a role at the earliest stage of lesion formation (Marik et al., 2007; Barnett et al., 2009), a more in-depth analysis showed that, although nodules localize on axons with impaired transport, they likely do not reflect an acute attack against myelinated axons because they occur in chronic but not an acute disease and because they can also be detected in the brains of patients with non-demyelinating diseases such as ischemic stroke (Michailidou et al., 2017). Therefore, it was concluded that it is unlikely that microglial nodules around C3d-coated axons drive the formation of new lesions but could represent a physiological mechanism to remove irreversibly damaged axons in chronic disease (Michailidou et al., 2017; **Figure 5B**).

In the gray matter, neurons and synapses could be potential hotspots of complement-mediated primary cytodegeneration (**Figure 5C**). Spurred by evidence implicating early complement components in the pruning of synapses during development (Stevens et al., 2007; Schafer et al., 2012), adulthood (Wang et al., 2020), normal aging (Stephan et al., 2013; see "Early Complement

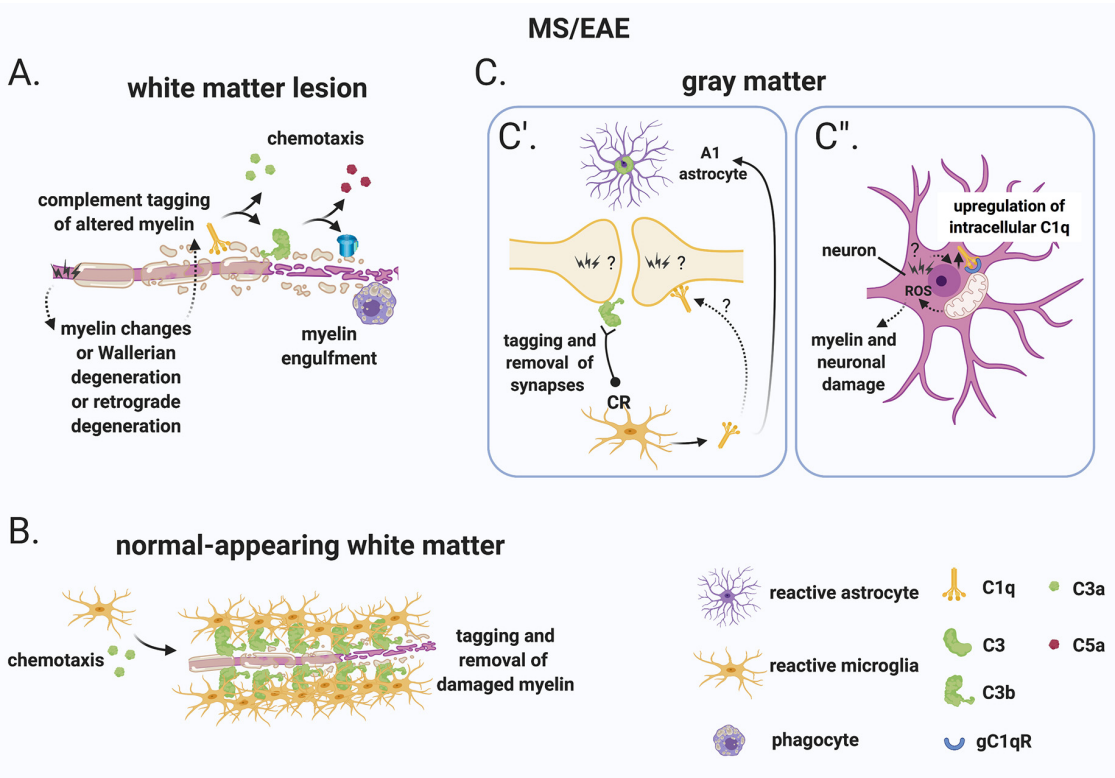

FIGURE 5 | A role for complement in the "inside-out" paradigm of demyelination and synapse loss in MS. **(A)** The "inside-out" model argues that a so far unknown primary cytodegenerative event leads to myelin changes or Wallerian degeneration or retrograde degeneration which exposes altered highly antigenic myelin epitopes. In this scenario, the complement system would participate in the secondary autoimmune and inflammatory response by tagging myelin and promoting its clearance by phagocytes. **(B)** In the normal-appearing white matter, complement (particularly activated C3) tags axons that may be damaged as a result of Wallerian degeneration or retrograde degeneration. C3-tagged altered myelin is then removed by clusters of microglia (also called microglial nodules). **(C)** In the gray matter, neurons and synapses could be potential hotspots of complement-mediated primary cytodegeneration. **(C′)** Synapses (of the visual thalamus) are tagged by C3b and engulfed by microglia. Tagging of synapses by C1q has also been reported in the MS hippocampus but its role in the early engulfment of synapses has not yet been investigated. Also, microglia-derived C1q (together with IL-1α and TNF) induces a subtype of $C3^+$ reactive astrocytes, termed A1 astrocytes, which are neurotoxic. **(C″)** Neurons upregulate C1q by an unknown trigger. C1q can then potentially bind to its receptor (gC1qR) on the surface of mitochondria triggering the production of ROS which in turn can induce myelin and neuronal damage. Abbreviations: ROS, reactive oxygen species.

Components in the Developing, Adult, and Normal Aging CNS" section) and neurodegeneration (Hong et al., 2016; see also "Early Complement Components in Neurodegeneration" section), studies of early complement components in the myelinated and demyelinated MS hippocampus were performed; these showed that C1q and C3d are deposited at synapses that localize within microglial processes and lysosomes particularly in the CA2/CA3 hippocampal subfield, supporting a role for complement-mediated elimination and degradation of synapses by microglia (Michailidou et al., 2015). A more recent study showed that in the $MOG_{35–55}$ EAE mouse model of demyelination synapses are reduced while C1q and C3 are both upregulated in the CA1 hippocampal subfield at 28–30 days post-immunization. Notably, C3 deletion rescued synapse loss and improved clinical outcomes (Hammond et al., 2020). However, since complement C3 plays a broader role in the dynamics of EAE, it is difficult to ascribe the protective effects of C3 deletion to a local role of C3 within the hippocampus. Studies at early (pre-onset) stages of EAE or in non-immune mediated models of hippocampal demyelination (such as the cuprizone model) may be more informative at pinpointing the kinetics of complement-dependent pruning of synapses in MS. Indeed, a recent study analyzing brain tissue at the onset of moderate clinical symptoms of models of demyelination showed that synaptic material is tagged by C3 (but not by C1q) and is engulfed by microglia in the retinogeniculate system of models of demyelination (Werneburg et al., 2020), dissociating complement activation at synapses from immune-mediated demyelination in this model (**Figure 5C'**).

What initiates complement activation in the MS hippocampus and visual thalamus is unknown but is an important question. The observations that neuronal production of C1q has been observed in the MS hippocampus (Michailidou et al., 2015) and in the retinogeniculate system (Bialas and Stevens, 2013) would suggest that the signal to eliminate synapses originates from the target neuron. The mechanisms responsible for the recruitment of C1q to synapses also remains unknown. Antibodies are the obvious candidate, but deposits of immunoglobulins were not detected in the MS hippocampus. An alternative target of C1q is an apoptotic signal on the surface of dead or dying cells, such as the previously identified ceramide transporter protein (Bode et al., 2014) or the externalization of phosphatidylserine (PS; Scott-Hewitt et al., 2020). Exposed PS have been shown in the cortex of adult mice in isolated synaptosomes that are also tagged by C1q (Györffy et al., 2018) and have also been observed *in vivo* at presynaptic inputs in the hippocampus and in the dLGN of developing mice (Scott-Hewitt et al., 2020). During development, deletion of C1q results in increased PS-labeled presynaptic inputs and decreased microglial engulfment of PS-labeled elements (Scott-Hewitt et al., 2020), implicating C1q in the elimination of PS-labeled synapses. It is likely that in this context, PS-labeled elements are engulfed by microglia *via* the phagocytic receptor TREM2 since, in hippocampal neuron and microglia co-cultures, synapse elimination can be partially prevented by deleting TREM2 on microglia (Scott-Hewitt et al., 2020). While this evidence point towards a potential role for an apoptotic signal in the C1q-mediated elimination of synapses which could be of significance in the MS brain, MS neurons are quite resistant to cell death (Dutta et al., 2011) which argues against C1q-driven opsonization of apoptotic neurons. Notably, local pruning of dendritic spines can occur by a caspase-3–dependent mechanism without inducing apoptosis (Ertürk et al., 2014). This evidence suggests that programmed cell death may be initiated but spatially restricted by inhibitors that allow the elimination of synapses without killing the target neuron. This pathway of local pruning of dendrites is initiated by the mitochondrial production of reactive oxygen species (ROS) and/or by the activation of the N-methyl-D-aspartate receptor (NMDA) receptors locally in dendrites (Ertürk et al., 2014). Both mitochondrial oxidative stress (Mahad et al., 2009; Witte et al., 2009; Fischer et al., 2012) and changes in the glutamate neurotransmitter system (Dutta et al., 2011) have been reported in MS. Based on these findings it is tempting to speculate that similar pathways may regulate synaptic pruning in the MS hippocampus. Also, poor tissue oxygenation (hypoxia) and "virtual hypoxia" could cause axonal changes that could potentially be targeted by the complement system. Hypoxia could arise partly from narrow-end arteries and arterioles that are vulnerable to oxygen desaturation or from veins that can deplete oxygen from the surrounding tissue (Martinez Sosa and Smith, 2017). "Virtual hypoxia" could arise from increased energy demand of demyelinated axons to generate action potentials and reduced axonal ATP production (Trapp and Stys, 2009).

In the context of the "inside-out" paradigm, a potential mechanism that has been suggested as a trigger of cytodegeneration is a putative deficiency of copper ions in the MS-affected CNS, with copper deficiency promoting demyelination and loss of myelinating oligodendroglia. This hypothesis originates from observations in the cuprizone model of demyelination, where the copper chelator cuprizone, results in varying degrees of oligodendroglial damage and demyelination in the CNS, with limited inflammation (Kipp et al., 2009). Since copper ions are known to desensitize NMDA receptors (Stys et al., 2012a; You et al., 2012), and in addition to neurons, oligodendrocytes and the myelin sheath also express NMDARs (Karadottir et al., 2005; Micu et al., 2006), it was suggested that dysregulation of copper homeostasis may result in chronic overactivation of these receptors, leading to excitotoxicity (Stys et al., 2012b). In this setting, it is tempting to speculate that the elimination of synapses by complement may be initially a protective mechanism that lowers excessive excitatory activity since such excitatory activity can eventually lead to pathological synaptic alterations. This hypothesis, however, remains to be tested.

INTRACELLULAR C1q IN THE CNS—"INSIDE THE INSIDE"

As mentioned, C1q is at least partially produced by cortical neurons in MS (Michailidou et al., 2015; Watkins et al., 2016) and neuronal production of C1q has also been observed in the retinogeniculate system (Bialas and Stevens, 2013). Intracellular C1q is emerging as an important modulator of cell metabolism particularly by acting on mitochondrial activity. For example, intracellular C1q can be recognized by the mitochondrially

expressed receptor gC1qR (Dedio et al., 1998) and was shown to drive mitochondrial production of ROS and subsequent neuronal death during hypoxia-mediated damage (Ten et al., 2010), which have been shown to occur in MS lesions (Mahad et al., 2009; Trapp and Stys, 2009; Witte et al., 2009; Fischer et al., 2012; Martinez Sosa and Smith, 2017).

ROS are chemically reactive species that can mediate demyelination and neurodegeneration in the MS-affected CNS. While in MS lesions the primary source of oxygen and nitric oxide radicals has been attributed to the oxidative burst induced in activated microglia and macrophages in the course of inflammation (Liu et al., 2001; Marik et al., 2007; Gray et al., 2008; Zeis et al., 2009; Fischer et al., 2012, 2013; Zrzavy et al., 2017), it remains possible that ROS also originates within neurons. Evidence of oxidative injury has been found in active MS lesions and includes oxidized lipids and proteins, as well as nitrosylated epitopes (Vladimirova et al., 1998; Bizzozero et al., 2005; Zeis et al., 2009). This is particularly evident in degenerating neurons and axons as well as in oligodendrocytes dying by apoptosis (Haider et al., 2011). Oxygen and nitric oxide radicals can also induce mitochondrial injury by disrupting mitochondrial enzyme function, by modifying mitochondrial proteins and accelerating their degradation. Free radicals can also interfere with *de novo* synthesis of respiratory chain components and can directly induce mitochondrial DNA damage (Bolaños et al., 1997; Smith et al., 1999). Therefore, the role of intracellular C1q in driving the production of ROS is consistent with the evidence of ROS and ROS-mediated injury in MS tissue.

In addition to C1q, C1q-TNF family proteins [also known as C1q-TNF-related protein (CTRP) family] play many roles in both immunity and metabolism. These molecules are structurally related to both C1q and TNF and form hybrid proteins (recently reviewed in Schaffler and Buechler, 2012). The C1q-TNF-CTRP family member, CTRP3, negatively regulates lipid metabolism by downregulating PPAR-γ and C/EBPα in adipocytes (Nishimoto et al., 2017), it has also been shown to mediate mitochondrial ROS production in smooth muscle cells (Feng et al., 2016), and it protects mesenchymal stem cells from ischemia-induced apoptosis *via* activation of the PI3K/Akt pathway (Hou et al., 2014). Importantly, CTRP3 knockdown by siRNA in bone marrow-derived mesenchymal stem cells proved that intracellular and/or autocrine C1q synthesis rather than systemic production was important for its function (Hou et al., 2014). While altered lipid metabolism (Kooij et al., 2019), mitochondrial ROS production (see above), and apoptosis (Lucchinetti et al., 2000) occur in MS, whether intracellular C1q plays a role in the ROS-mediated damage observed in the MS-affected CNS remains to be investigated (**Figure 5C″**).

APPROACHES TO THERAPIES TARGETING THE COMPLEMENT SYSTEM IN MS

Regardless of the modality in which the complement system is involved in MS, either as part of a primary autoimmune attack to the CNS (outside-in) or as part of a secondary event triggered by primary changes in the CNS (inside-out), uncontrolled activation can ultimately exacerbate an injury. Thus inhibition of complement may be protective. Identification of MS patients that may benefit from complement therapy, or pin-pointing a time-frame when complement therapy may be appropriate, would be invaluable in guiding clinicians to decide whether and when an anti-complement drug might be effective in a given patient. Measuring complement may also aid diagnosis, assessment of prognosis, and help in the monitoring of treatment response in this complex and heterogeneous disorder. Besides, although not reviewed here in detail, complement can also exert protective functions in the brain (reviewed in Ingram et al., 2009). Therefore, targeting complement-mediated injurious signals while maintaining potential protective roles, would have to be taken into account when designing an appropriate complement therapy.

Various complement proteins have also been considered as biomarkers of disease activity. One challenge in the identification of complement as a serological or CSF biomarker is the fact that complement components are acute-phase proteins whose synthesis and consumption are both increased in response to inflammation. As a consequence, the serum and CSF levels of a given complement component will be influenced by bouts of active inflammation, including infection. Nevertheless, while earlier studies showed inconsistent results (Jans et al., 1984; Jongen et al., 2000), more recent analysis on well-powered cohorts stratified based on clinical course and compared to age-matched controls have shown more consistent outcome for levels of complement components, activation products and regulators in serum and CSF (Ingram et al., 2010a,b, 2012). Systemic complement profiling has shown increased plasma levels of C3, C4, C4a, C1 inhibitor, and factor H, while levels of the terminal component C9 were reduced in MS patients compared with controls. Importantly, combined profiling of these analytes produced a statistical model with a predictive value of 97% for MS and 73% for clinical relapse when combined with selected demographic data (Ingram et al., 2012). Interestingly, plasma C4a levels were found to be raised only in acute relapse, decreasing over 2 months (Ingram et al., 2010b) and serum factor H levels were capable of distinguishing secondary progressive from relapsing-remitting disease (excluding patients in clinical relapse) with a sensitivity of 89.41%, a specificity of 69.47% and a positive predictive value of 72.38% (Ingram et al., 2010a). Therefore, specific complement components may be an effective indicator of progression or relapse and accessible biomarker to stratify patients, providing objective evidence to help guide therapeutic decisions.

While there is a growing complement therapeutics industry with many new emerging drugs (Carpanini et al., 2019), to date CNS targets have been largely ignored. Drug delivery across the BBB is a major challenge that needs to be considered when designing CNS-directed therapeutics. Perhaps therapies should target areas of pathology, as described for the fusion proteins linking CR2 (localizes to C3 activation products in tissues) with a complement regulator (see Werneburg et al., 2020), or they should target complexes, for example, TCC/MAC, which

is found only in areas of pathology (reviewed in Morgan and Harris, 2015).

Although much remains to be clarified, targeting specific effector pathways of complement in (a subgroup of) patients at a crucial time(s) during the disease course could be a useful therapeutic approach in the future.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, while it is clear that the complement system is involved in the pathology of the MS-affected CNS, the pathways that the complement system engages to aid in the clearance of myelin or to shape synaptic circuits in various regions of the MS brain can differ substantially. Increasing awareness of the distinct roles of complement components in normal brain development and in neurodegenerative disorders may guide our understanding of similar pathological processes in MS. Understanding the relative contribution of serum-derived complement proteins vs. those produced locally by brain resident cells across MS disease stages and how to complement gene expression is regulated in the brain will be important questions for future research. For example, conditional knock out mice in which a particular complement gene can be inactivated in specific cell types of the brain could help tease apart the contributions of complement derived from brain-resident cells vs. that derived from the periphery. Also, viral vector approaches that enable the expression of complement regulators at targeted surfaces within the brain could help test the effect of local complement activation while maintaining intact peripheral complement functions. As a complementary approach, bone marrow chimeras or targeted inhibition of hepatic source of complement could help modulate peripheral complement while maintaining intact complement produced by cells within the brain. The added knowledge may help answer the question of whether MS is initiated by "outside-in" or "inside out" disease mechanisms. The current knowledge is equally consistent with either paradigm, and it is likely that both mechanisms jointly contribute to the variable course of MS.

AUTHOR CONTRIBUTIONS

VR, JLG and BPM wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Absinta, M., Vuolo, L., Rao, A., Nair, G., Sati, P., Cortese, I. C., et al. (2015). Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis. *Neurology* 85, 18–28. doi: 10.1212/WNL.0000000000001587

Adams, R. A., Bauer, J., Flick, M. J., Sikorski, S. L., Nuriel, T., Lassmann, H., et al. (2007). The fibrin-derived $\gamma^{377-395}$ peptide inhibits microglia activation and suppresses relapsing paralysis in central nervous system autoimmune disease. *J. Exp. Med.* 204, 571–582. doi: 10.1084/jem.20061931

Adams, R. A., Passino, M., Sachs, B. D., Nuriel, T., and Akassoglou, K. (2004). Fibrin mechanisms and functions in nervous system pathology. *Mol. Interv.* 4, 163–176. doi: 10.1124/mi.4.3.6

Akassoglou, K., Adams, R. A., Bauer, J., Mercado, P., Tseveleki, V., Lassmann, H., et al. (2004). Fibrin depletion decreases inflammation and delays the onset of demyelination in a tumor necrosis factor transgenic mouse model for multiple sclerosis. *Proc. Natl. Acad. Sci. U S A* 101, 6698–6703. doi: 10.1073/pnas.0303859101

Akassoglou, K., and Strickland, S. (2002). Nervous system pathology: the fibrin perspective. *Biol. Chem.* 383, 37–45. doi: 10.1515/BC.2002.004

Amara, U., Flierl, M. A., Rittirsch, D., Klos, A., Chen, H., Acker, B., et al. (2010). Molecular intercommunication between the complement and coagulation systems. *J. Immunol.* 185, 5628–5636. doi: 10.4049/jimmunol.0903678

Anderson, A. J., Robert, S., Huang, W., Young, W., and Cotman, C. W. (2004). Activation of complement pathways after contusion-induced spinal cord injury. *J. Neurotrauma* 21, 1831–1846. doi: 10.1089/neu.2004.21.1831

Arbore, G., West, E. E., Rahman, J., Le Friec, G., Niyonzima, N., Pirooznia, M., et al. (2018). Complement receptor CD46 co-stimulates optimal human $CD8^+$ T cell effector function *via* fatty acid metabolism. *Nat. Commun.* 9:4186. doi: 10.1038/s41467-018-06706-z

Astier, A. L., Meiffren, G., Freeman, S., and Hafler, D. A. (2006). Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. *J. Clin. Invest.* 116, 3252–3257. doi: 10.1172/JCI29251

Baker, D., O'Neill, J. K., Gschmeissner, S. E., Wilcox, C. E., Butter, C., and Turk, J. L. (1990). Induction of chronic relapsing experimental allergic encephalomyelitis in Biozzi mice. *J. Neuroimmunol.* 28, 261–270. doi: 10.1016/0165-5728(90)90019-j

Barnett, M. H., Parratt, J. D., Cho, E.-S., and Prineas, J. W. (2009). Immunoglobulins and complement in postmortem multiple sclerosis tissue. *Ann. Neurol.* 65, 32–46. doi: 10.1002/ana.21524

Barnett, M. H., and Prineas, J. W. (2004). Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol.* 55, 458–468. doi: 10.1002/ana.20016

Berger, T., and Reindl, M. (2007). Multiple sclerosis: disease biomarkers as indicated by pathophysiology. *J. Neurol. Sci.* 259, 21–26. doi: 10.1016/j.jns.2006.05.070

Bialas, A. R., and Stevens, B. (2013). TGF-β signaling regulates neuronal C1q expression and developmental synaptic refinement. *Nat. Neurosci.* 16, 1773–1782. doi: 10.1038/nn.3560

Bizzozero, O. A., DeJesus, G., Callahan, K., and Pastuszyn, A. (2005). Elevated protein carbonylation in the brain white matter and gray matter of patients with multiple sclerosis. *J. Neurosci. Res.* 81, 687–695. doi: 10.1002/jnr.20587

Bjartmar, L., Huberman, A. D., Ullian, E. M., Rentería, R. C., Liu, X., Xu, W., et al. (2006). Neuronal pentraxins mediate synaptic refinement in the developing visual system. *J. Neurosci.* 26, 6269–6281. doi: 10.1523/JNEUROSCI.4212-05.2006

Bø, L., Vedeler, C. A., Nyland, H. I., Trapp, B. D., and Mørk, S. J. (2003). Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J. Neuropathol. Exp. Neurol.* 62, 723–732. doi: 10.1093/jnen/62.7.723

Bode, G. H., Losen, M., Buurman, W. A., Veerhuis, R., Molenaar, P. C., Steinbusch, H. W., et al. (2014). Complement activation by ceramide transporter proteins. *J. Immunol.* 192, 1154–1161. doi: 10.4049/jimmunol.1301673

Bolaños, J. P., Almeida, A., Stewart, V., Peuchen, S., Land, J. M., Clark, J. B., et al. (1997). Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J. Neurochem.* 68, 2227–2240. doi: 10.1046/j.1471-4159.1997.68062227.x

Bonifati, D. M., and Kishore, U. (2007). Role of complement in neurodegeneration and neuroinflammation. *Mol. Immunol.* 44, 999–1010. doi: 10.1016/j.molimm.2006.03.007

Breij, E. C., Brink, B. P., Veerhuis, R., van den Berg, C., Vloet, R., Yan, R., et al. (2008). Homogeneity of active demyelinating lesions in established multiple sclerosis. *Ann. Neurol.* 63, 16–25. doi: 10.1002/ana.21311

Brennan, F. H., Lee, J. D., Ruitenberg, M. J., and Woodruff, T. M. (2016). Therapeutic targeting of complement to modify disease course and improve outcomes in neurological conditions. *Semin. Immunol.* 28, 292–308. doi: 10.1016/j.smim.2016.03.015

Brink, B. P., Veerhuis, R., Breij, E. C., van der Valk, P., Dijkstra, C. D., and Bo, L. (2005). The pathology of multiple sclerosis is location-dependent: no significant complement activation is detected in purely cortical lesions. *J. Neuropathol. Exp. Neurol.* 64, 147–155. doi: 10.1093/jnen/64.2.147

Brownell, B., and Hughes, J. T. (1962). The distribution of plaques in the cerebrum in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 25, 315–320. doi: 10.1136/jnnp.25.4.315

Butovsky, O., Jedrychowski, M. P., Moore, C. S., Cialic, R., Lanser, A. J., Gabriely, G., et al. (2014). Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat. Neurosci.* 17, 131–143. doi: 10.1038/nn.3599

Butter, C., Baker, D., O'Neill, J. K., and Turk, J. L. (1991). Mononuclear cell trafficking and plasma protein extravasation into the CNS during chronic relapsing experimental allergic encephalomyelitis in Biozzi AB/H mice. *J. Neurol. Sci.* 104, 9–12. doi: 10.1016/0022-510x(91)90209-p

Calabrese, M., Filippi, M., and Gallo, P. (2010). Cortical lesions in multiple sclerosis. *Nat. Rev. Neurol.* 6, 438–444. doi: 10.1038/nrneurol.2010.93

Calabrese, M., Poretto, V., Favaretto, A., Alessio, S., Bernardi, V., Romualdi, C., et al. (2012). Cortical lesion load associates with progression of disability in multiple sclerosis. *Brain* 135, 2952–2961. doi: 10.1093/brain/aws246

Carpanini, S. M., Torvell, M., and Morgan, B. P. (2019). Therapeutic inhibition of the complement system in diseases of the central nervous system. *Front. Immunol.* 10:362. doi: 10.3389/fimmu.2019.00362

Chao, Y., Wong, S. C., and Tan, E. K. (2014). Evidence of inflammatory system involvement in Parkinson's disease. *Biomed. Res. Int.* 2014:308654. doi: 10.1155/2014/308654

Charcot, J. M. (1880). *Lecons sur les Maladies du Systeme Nerveux Faites a la Salpetriere.* 4th Edn. Paris: Adrien Delahaye et E. Lecrosnier.

Chard, D., and Miller, D. (2009). Grey matter pathology in clinically early multiple sclerosis: evidence from magnetic resonance imaging. *J. Neurol. Sci.* 282, 5–11. doi: 10.1016/j.jns.2009.01.012

Choi, S. R., Howell, O. W., Carassiti, D., Magliozzi, R., Gveric, D., Muraro, P. A., et al. (2012). Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. *Brain* 135, 2925–2937. doi: 10.1093/brain/aws189

Ciotti, J. R., and Cross, A. H. (2018). Disease-modifying treatment in progressive multiple sclerosis. *Curr. Treat. Options Neurol.* 20:12. doi: 10.1007/s11940-018-0496-3

Compston, D. A., Morgan, B. P., Campbell, A. K., Wilkins, P., Cole, G., Thomas, N. D., et al. (1989). Immunocytochemical localization of the terminal complement complex in multiple sclerosis. *Neuropathol. Appl. Neurobiol.* 15, 307–316. doi: 10.1111/j.1365-2990.1989.tb01231.x

Corriveau, R. A., Huh, G. S., and Shatz, C. J. (1998). Regulation of class I MHC gene expression in the developing and mature CNS by neural activity. *Neuron* 21, 505–520. doi: 10.1016/s0896-6273(00)80562-0

Datwani, A., McConnell, M. J., Kanold, P. O., Micheva, K. D., Busse, B., Shamloo, M., et al. (2009). Classical MHCI molecules regulate retinogeniculate refinement and limit ocular dominance plasticity. *Neuron* 64, 463–470. doi: 10.1016/j.neuron.2009.10.015

Davalos, D., Ryu, J. K., Merlini, M., Baeten, K. M., Le Moan, N., Petersen, M. A., et al. (2012). Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. *Nat. Commun.* 3:1227. doi: 10.1038/ncomms2230

Dawson, J. D. (1916). The histology of disseminated sclerosis. *Trans. R. Soc. Edinb.* 50, 517–740. doi: 10.1017/S0080456800027174

Dedio, J., Jahnen-Dechent, W., Bachmann, M., and Muller-Esterl, W. (1998). The multiligand-binding protein gC1qR, putative C1q receptor, is a mitochondrial protein. *J. Immunol.* 160, 3534–3542.

Dempsey, P. W., Allison, M. E., Akkaraju, S., Goodnow, C. C., and Fearon, D. T. (1996). C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science* 271, 348–350. doi: 10.1126/science.271.5247.348

Dutta, R., Chang, A., Doud, M. K., Kidd, G. J., Ribaudo, M. V., Young, E. A., et al. (2011). Demyelination causes synaptic alterations in hippocampi from multiple sclerosis patients. *Ann. Neurol.* 69, 445–454. doi: 10.1002/ana.22337

Engelhardt, B., Carare, R. O., Bechmann, I., Flugel, A., Laman, J. D., and Weller, R. O. (2016). Vascular, glial and lymphatic immune gateways of the central nervous system. *Acta Neuropathol.* 132, 317–338. doi: 10.1007/s00401-016-1606-5

Engelhardt, B., and Coisne, C. (2011). Fluids and barriers of the CNS establish immune privilege by confining immune surveillance to a two-walled castle moat surrounding the CNS castle. *Fluids Barriers CNS* 8:4. doi: 10.1186/2045-8118-8-4

Ertürk, A., Wang, Y., and Sheng, M. (2014). Local pruning of dendrites and spines by caspase-3-dependent and proteasome-limited mechanisms. *J. Neurosci.* 34, 1672–1688. doi: 10.1523/JNEUROSCI.3121-13.2014

Feger, U., Luther, C., Poeschel, S., Melms, A., Tolosa, E., and Wiendl, H. (2007). Increased frequency of $CD4^+$ $CD25^+$ regulatory T cells in the cerebrospinal fluid but not in the blood of multiple sclerosis patients. *Clin. Exp. Immunol.* 147, 412–418. doi: 10.1111/j.1365-2249.2006.03271.x

Feng, H., Wang, J.-Y., Zheng, M., Zhang, C.-L., An, Y.-M., Li, L., et al. (2016). CTRP3 promotes energy production by inducing mitochondrial ROS and up-expression of PGC-1α in vascular smooth muscle cells. *Exp. Cell Res.* 341, 177–186. doi: 10.1016/j.yexcr.2016.02.001

Filipello, F., Morini, R., Corradini, I., Zerbi, V., Canzi, A., Michalski, B., et al. (2018). The microglial innate immune receptor TREM2 is required for synapse elimination and normal brain connectivity. *Immunity* 48, 979.e8–991.e8. doi: 10.1016/j.immuni.2018.04.016

Fischer, M. T., Sharma, R., Lim, J. L., Haider, L., Frischer, J. M., Drexhage, J., et al. (2012). NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. *Brain* 135, 886–899. doi: 10.1093/brain/aws012

Fischer, M. T., Wimmer, I., Hoftberger, R., Gerlach, S., Haider, L., Zrzavy, T., et al. (2013). Disease-specific molecular events in cortical multiple sclerosis lesions. *Brain* 136, 1799–1815. doi: 10.1093/brain/awt110

Fitzgerald, K. C., Kim, K., Smith, M. D., Aston, S. A., Fioravante, N., Rothman, A. M., et al. (2019). Early complement genes are associated with visual system degeneration in multiple sclerosis. *Brain* 142, 2722–2736. doi: 10.1093/brain/awz188

Fluiter, K., Opperhuizen, A. L., Morgan, B. P., Baas, F., and Ramaglia, V. (2014). Inhibition of the membrane attack complex of the complement system reduces secondary neuroaxonal loss and promotes neurologic recovery after traumatic brain injury in mice. *J. Immunol.* 192, 2339–2348. doi: 10.4049/jimmunol.1302793

Fonseca, M. I., Chu, S.-H., Berci, A. M., Benoit, M. E., Peters, D. G., Kimura, Y., et al. (2011). Contribution of complement activation pathways to neuropathology differs among mouse models of Alzheimer's disease. *J. Neuroinflammation* 8:4. doi: 10.1186/1742-2094-8-4

Fonseca, M. I., Chu, S.-H., Hernandez, M. X., Fang, M. J., Modarresi, L., Selvan, P., et al. (2017). Cell-specific deletion of C1qa identifies microglia as the dominant source of C1q in mouse brain. *J. Neuroinflammation* 14:48. doi: 10.1186/s12974-017-0814-9

Fonseca, M. I., Zhou, J., Botto, M., and Tenner, A. J. (2004). Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. *J. Neurosci.* 24, 6457–6465. doi: 10.1523/JNEUROSCI.0901-04.2004

Fransen, N. L., Hsiao, C.-C., van der Poel, M., Engelenburg, H. J., Verdaasdonk, K., Vincenten, M. C. J., et al. (2020). Tissue-resident memory T cells invade the brain parenchyma in multiple sclerosis white matter lesions. *Brain* 143, 1714–1730. doi: 10.1093/brain/awaa117

Fraser, D. A., Pisalyaput, K., and Tenner, A. J. (2010). C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. *J. Neurochem.* 112, 733–743. doi: 10.1111/j.1471-4159.2009.06494.x

Frischer, J. M., Bramow, S., Dal-Bianco, A., Lucchinetti, C. F., Rauschka, H., Schmidbauer, M., et al. (2009). The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 132, 1175–1189. doi: 10.1093/brain/awp070

Frischer, J. M., Weigand, S. D., Guo, Y., Kale, N., Parisi, J. E., Pirko, I., et al. (2015). Clinical and pathological insights into the dynamic nature of the white matter multiple sclerosis plaque. *Ann. Neurol.* 78, 710–721. doi: 10.1002/ana.24497

Fu, H., Liu, B., Frost, J. L., Hong, S., Jin, M., Ostaszewski, B., et al. (2012). Complement component C3 and complement receptor type 3 contribute to the phagocytosis and clearance of fibrillar Aβ by microglia. *Glia* 60, 993–1003. doi: 10.1002/glia.22331

Fu, M., and Zuo, Y. (2011). Experience-dependent structural plasticity in the cortex. *Trends Neurosci.* 34, 177–187. doi: 10.1016/j.tins.2011.02.001

Galicia, G., Boulianne, B., Pikor, N., Martin, A., and Gommerman, J. L. (2013). Secondary B cell receptor diversification is necessary for T cell mediated neuroinflammation during experimental autoimmune encephalomyelitis. *PLoS One* 8:e61478. doi: 10.1371/journal.pone.0061478

Galli, E., Hartmann, F. J., Schreiner, B., Ingelfinger, F., Arvaniti, E., Diebold, M., et al. (2019). GM-CSF and CXCR4 define a T helper cell signature in multiple sclerosis. *Nat. Med.* 25, 1290–1300. doi: 10.1038/s41591-019-0521-4

Galvan, M. D., Luchetti, S., Burgos, A. M., Nguyen, H. X., Hooshmand, M. J., Hamers, F. P., et al. (2008). Deficiency in complement C1q improves histological and functional locomotor outcome after spinal cord injury. *J. Neurosci.* 28, 13876–13888. doi: 10.1523/JNEUROSCI.2823-08.2008

Gardner, C., Magliozzi, R., Durrenberger, P. F., Howell, O. W., Rundle, J., and Reynolds, R. (2013). Cortical grey matter demyelination can be induced by elevated pro-inflammatory cytokines in the subarachnoid space of MOG-immunized rats. *Brain* 136, 3596–3608. doi: 10.1093/brain/awt279

Gasque, P., Chan, P., Fontaine, M., Ischenko, A., Lamacz, M., Gotze, O., et al. (1995). Identification and characterization of the complement C5a anaphylatoxin receptor on human astrocytes. *J. Immunol.* 155, 4882–4889.

Gasque, P., Chan, P., Mauger, C., Schouft, M. T., Singhrao, S., Dierich, M. P., et al. (1996). Identification and characterization of complement C3 receptors on human astrocytes. *J. Immunol.* 156, 2247–2255.

Gasque, P., Dean, Y. D., McGreal, E. P., VanBeek, J., and Morgan, B. P. (2000). Complement components of the innate immune system in health and disease in the CNS. *Immunopharmacology* 49, 171–186. doi: 10.1016/s0162-3109(00)80302-1

Gasque, P., Ischenko, A., Legoedec, J., Mauger, C., Schouft, M. T., and Fontaine, M. (1993). Expression of the complement classical pathway by human glioma in culture. A model for complement expression by nerve cells. *J. Biol. Chem.* 268, 25068–25074.

Gasque, P., Julen, N., Ischenko, A. M., Picot, C., Mauger, C., Chauzy, C., et al. (1992). Expression of complement components of the alternative pathway by glioma cell lines. *J. Immunol.* 149, 1381–1387.

Gasque, P., Singhrao, S. K., Neal, J. W., Gätze, O., and Morgan, B. P. (1997). Expression of the receptor for complement C5a (CD88) is up-regulated on reactive astrocytes, microglia, and endothelial cells in the inflamed human central nervous system. *Am. J. Pathol.* 150, 31–41.

Geurts, J. J., Bø, L., Roosendaal, S. D., Hazes, T., Daniels, R., Barkhof, F., et al. (2007). Extensive hippocampal demyelination in multiple sclerosis. *J. Neuropathol. Exp. Neurol.* 66, 819–827. doi: 10.1097/nen.0b013e3181461f54

Gold, S. M., Kern, K. C., O'Connor, M. F., Montag, M. J., Kim, A., Yoo, Y. S., et al. (2010). Smaller cornu ammonis 2-3/dentate gyrus volumes and elevated cortisol in multiple sclerosis patients with depressive symptoms. *Biol. Psychiatry* 68, 553–559. doi: 10.1016/j.biopsych.2010.04.025

Gold, S. M., O'Connor, M. F., Gill, R., Kern, K. C., Shi, Y., Henry, R. G., et al. (2014). Detection of altered hippocampal morphology in multiple sclerosis-associated depression using automated surface mesh modeling. *Hum. Brain Mapp.* 35, 30–37. doi: 10.1002/hbm.22154

Gray, E., Thomas, T. L., Betmouni, S., Scolding, N., and Love, S. (2008). Elevated activity and microglial expression of myeloperoxidase in demyelinated cerebral cortex in multiple sclerosis. *Brain Pathol.* 18, 86–95. doi: 10.1111/j.1750-3639.2007.00110.x

Györffy, B. A., Kun, J., Török, G., Bulyáki, E., Borhegyi, Z., Gulyássy, P., et al. (2018). Local apoptotic-like mechanisms underlie complement-mediated synaptic pruning. *Proc. Natl. Acad. Sci. U S A* 115, 6303–6308. doi: 10.1073/pnas.1722613115

Haider, L., Fischer, M. T., Frischer, J. M., Bauer, J., Hoftberger, R., Botond, G., et al. (2011). Oxidative damage in multiple sclerosis lesions. *Brain* 134, 1914–1924. doi: 10.1093/brain/awr128

Haider, L., Zrzavy, T., Hametner, S., Höftberger, R., Bagnato, F., Grabner, G., et al. (2016). The topograpy of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain* 139, 807–815. doi: 10.1093/brain/awv398

Hajishengallis, G., Reis, E. S., Mastellos, D. C., Ricklin, D., and Lambris, J. D. (2017). Novel mechanisms and functions of complement. *Nat. Immunol.* 18, 1288–1298. doi: 10.1038/ni.3858

Hammond, J. W., Bellizzi, M. J., Ware, C., Qiu, W. Q., Saminathan, P., Li, H., et al. (2020). Complement-dependent synapse loss and microgliosis in a mouse model of multiple sclerosis. *Brain Behav. Immun.* 87, 739–750. doi: 10.1016/j.bbi.2020.03.004

Harrison, D. M., Roy, S., Oh, J., Izbudak, I., Pham, D., Courtney, S., et al. (2015). Association of cortical lesion burden on 7-T magnetic resonance imaging with cognition and disability in multiple sclerosis. *JAMA Neurol.* 72, 1004–1012. doi: 10.1001/jamaneurol.2015.1241

Henderson, A. P., Barnett, M. H., Parratt, J. D., and Prineas, J. W. (2009). Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann. Neurol.* 66, 739–753. doi: 10.1002/ana.21800

Hong, S., Beja-Glasser, V. F., Nfonoyim, B. M., Frouin, A., Li, S., Ramakrishnan, S., et al. (2016). Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 352, 712–716. doi: 10.1126/science.aad8373

Hou, M., Liu, J., Liu, F., Liu, K., and Yu, B. (2014). C1q tumor necrosis factor-related protein-3 protects mesenchymal stem cells against hypoxia- and serum deprivation-induced apoptosis through the phosphoinositide 3-kinase/Akt pathway. *Int. J. Mol. Med.* 33, 97–104. doi: 10.3892/ijmm.2013.1550

Howell, G. R., Macalinao, D. G., Sousa, G. L., Walden, M., Soto, I., Kneeland, S. C., et al. (2011). Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma. *J. Clin. Invest.* 121, 1429–1444. doi: 10.1172/JCI44646

Howell, O. W., Reeves, C. A., Nicholas, R., Carassiti, D., Radotra, B., Gentleman, S. M., et al. (2011). Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. *Brain* 134, 2755–2771. doi: 10.1093/brain/awr182

Howell, O. W., Schulz-Trieglaff, E. K., Carassiti, D., Gentleman, S. M., Nicholas, R., Roncaroli, F., et al. (2015). Extensive grey matter pathology in the cerebellum in multiple sclerosis is linked to inflammation in the subarachnoid space. *Neuropathol. Appl. Neurobiol.* 41, 798–813. doi: 10.1111/nan.12199

Huber-Lang, M., Sarma, J. V., Zetoune, F. S., Rittirsch, D., Neff, T. A., McGuire, S. R., et al. (2006). Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* 12, 682–687. doi: 10.1038/nm1419

Ighani, M., Jonas, S., Izbudak, I., Choi, S., Lema-Dopico, A., Hua, J., et al. (2020). No association between cortical lesions and leptomeningeal enhancement on 7-Tesla MRI in multiple sclerosis. *Mult. Scler.* 26, 165–176. doi: 10.1177/1352458519876037

Ingram, G., Hakobyan, S., Hirst, C. L., Harris, C. L., Loveless, S., Mitchell, J. P., et al. (2012). Systemic complement profiling in multiple sclerosis as a biomarker of disease state. *Mult. Scler.* 18, 1401–1411. doi: 10.1177/1352458512438238

Ingram, G., Hakobyan, S., Hirst, C. L., Harris, C. L., Pickersgill, T. P., Cossburn, M. D., et al. (2010a). Complement regulator factor H as a serum biomarker of multiple sclerosis disease state. *Brain* 133, 1602–1611. doi: 10.1093/brain/awq085

Ingram, G., Hakobyan, S., Robertson, N. P., and Morgan, B. P. (2010b). Elevated plasma C4a levels in multiple sclerosis correlate with disease activity. *J. Neuroimmunol.* 223, 124–127. doi: 10.1016/j.jneuroim.2010.03.014

Ingram, G., Hakobyan, S., Robertson, N. P., and Morgan, B. P. (2009). Complement in multiple sclerosis: its role in disease and potential as a biomarker. *Clin. Exp. Immunol.* 155, 128–139. doi: 10.1111/j.1365-2249.2008.03830.x

Ingram, G., Loveless, S., Howell, O. W., Hakobyan, S., Dancey, B., Harris, C. L., et al. (2014). Complement activation in multiple sclerosis plaques: an immunohistochemical analysis. *Acta Neuropathol. Commun.* 2:53. doi: 10.1186/2051-5960-2-53

International Multiple Sclerosis Genetics Consortium. (2019). Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science* 365:eaav7188. doi: 10.1126/science.aav7188

Jackson, S. J., Lee, J., Nikodemova, M., Fabry, Z., and Duncan, I. D. (2009). Quantification of myelin and axon pathology during relapsing progressive experimental autoimmune encephalomyelitis in the Biozzi ABH mouse. *J. Neuropathol. Exp. Neurol.* 68, 616–625. doi: 10.1097/NEN.0b013e3181a41d23

Jans, H., Heltberg, A., Zeeberg, I., Kristensen, J. H., Fog, T., and Raun, N. E. (1984). Immune complexes and the complement factors C4 and C3 in cerebrospinal fluid and serum from patients with chronic progressive multiple sclerosis. *Acta Neurol. Scand.* 69, 34–38. doi: 10.1111/j.1600-0404.1984.tb07777.x

Jongen, P. J., Doesburg, W. H., Ibrahim-Stappers, J. L., Lemmens, W. A., Hommes, O. R., and Lamers, K. J. (2000). Cerebrospinal fluid C3 and C4 indexes in immunological disorders of the central nervous system. *Acta Neurol. Scand.* 101, 116–121. doi: 10.1034/j.1600-0404.2000.101002116.x

Joseph, F. G., Hirst, C. L., Pickersgill, T. P., Ben-Shlomo, Y., Robertson, N. P., and Scolding, N. J. (2009). CSF oligoclonal band status informs prognosis in multiple sclerosis: a case control study of 100 patients. *J. Neurol. Neurosurg. Psychiatry* 80, 292–296. doi: 10.1136/jnnp.2008.150896

Kaczorowski, S. L., Schiding, J. K., Toth, C. A., and Kochanek, P. M. (1995). Effect of soluble complement receptor-1 on neutrophil accumulation after traumatic brain injury in rats. *J. Cereb. Blood Flow Metab.* 15, 860–864. doi: 10.1038/jcbfm.1995.107

Karadottir, R., Cavelier, P., Bergersen, L. H., and Attwell, D. (2005). NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. *Nature* 438, 1162–1166. doi: 10.1038/nature04302

Kaskow, B. J., and Baecher-Allan, C. (2018). Effector T cells in multiple sclerosis. *Cold Spring Harb. Perspect. Med.* 8:a029025. doi: 10.1101/cshperspect.a029025

Kebir, H., Kreymborg, K., Ifergan, I., Dodelet-Devillers, A., Cayrol, R., Bernard, M., et al. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat. Med.* 13, 1173–1175. doi: 10.1038/nm1651

Kidd, D., Barkhof, F., McConnell, R., Algra, P. R., Allen, I. V., and Revesz, T. (1999). Cortical lesions in multiple sclerosis. *Brain* 122, 17–26. doi: 10.1093/brain/122.1.17

Kipp, M., Clarner, T., Dang, J., Copray, S., and Beyer, C. (2009). The cuprizone animal model: new insights into an old story. *Acta Neuropathol.* 118, 723–736. doi: 10.1007/s00401-009-0591-3

Kooi, E. J., Geurts, J. J., van Horssen, J., Bo, L., and van der Valk, P. (2009). Meningeal inflammation is not associated with cortical demyelination in chronic multiple sclerosis. *J. Neuropathol. Exp. Neurol.* 68, 1021–1028. doi: 10.1097/NEN.0b013e3181b4bf8f

Kooij, G., Derada Troletti, C., Leuti, A., Norris, P. C., Riley, I., Albanese, M., et al. (2019). Specialized pro-resolving lipid mediators are differentially altered in peripheral blood of patients with multiple sclerosis and attenuate monocyte and blood-brain barrier dysfunction. *Haematologica* 105, 2056–2070. doi: 10.3324/haematol.2019.219519

Kuhlmann, T., Ludwin, S., Prat, A., Antel, J., Bruck, W., and Lassmann, H. (2017). An updated histological classification system for multiple sclerosis lesions. *Acta Neuropathol.* 133, 13–24. doi: 10.1007/s00401-016-1653-y

Kutzelnigg, A., and Lassmann, H. (2014). Pathology of multiple sclerosis and related inflammatory demyelinating diseases. *Handb. Clin. Neurol.* 122, 15–58. doi: 10.1016/B978-0-444-52001-2.00002-9

Kutzelnigg, A., Lucchinetti, C. F., Stadelmann, C., Bruck, W., Rauschka, H., Bergmann, M., et al. (2005). Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 128, 2705–2712. doi: 10.1093/brain/awh641

Lagumersindez-Denis, N., Wrzos, C., Mack, M., Winkler, A., van der Meer, F., Reinert, M. C., et al. (2017). Differential contribution of immune effector mechanisms to cortical demyelination in multiple sclerosis. *Acta Neuropathol.* 134, 15–34. doi: 10.1007/s00401-017-1706-x

Lassmann, H., Bruck, W., and Lucchinetti, C. F. (2007). The immunopathology of multiple sclerosis: an overview. *Brain Pathol.* 17, 210–218. doi: 10.1111/j.1750-3639.2007.00064.x

Lee, J. D., Coulthard, L. G., and Woodruff, T. M. (2019). Complement dysregulation in the central nervous system during development and disease. *Semin. Immunol.* 45:101340. doi: 10.1016/j.smim.2019.101340

Lehrman, E. K., Wilton, D. K., Litvina, E. Y., Welsh, C. A., Chang, S. T., Frouin, A., et al. (2018). CD47 protects synapses from excess microglia-mediated pruning during development. *Neuron* 100, 120.e6–134.e6. doi: 10.1016/j.neuron.2018.09.017

Leinhase, I., Rozanski, M., Harhausen, D., Thurman, J. M., Schmidt, O. I., Hossini, A. M., et al. (2007). Inhibition of the alternative complement activation pathway in traumatic brain injury by a monoclonal anti-factor B antibody: a randomized placebo-controlled study in mice. *J. Neuroinflammation* 4:13. doi: 10.1186/1742-2094-4-13

Leinhase, I., Schmidt, O. I., Thurman, J. M., Hossini, A. M., Rozanski, M., Taha, M. E., et al. (2006). Pharmacological complement inhibition at the C3 convertase level promotes neuronal survival, neuroprotective intracerebral gene expression and neurological outcome after traumatic brain injury. *Exp. Neurol.* 199, 454–464. doi: 10.1016/j.expneurol.2006.01.033

Levi-Strauss, M., and Mallat, M. (1987). Primary cultures of murine astrocytes produce C3 and factor B, two components of the alternative pathway of complement activation. *J. Immunol.* 139, 2361–2366.

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481–487. doi: 10.1038/nature21029

Liszewski, M. K., Kolev, M., Le Friec, G., Leung, M., Bertram, P. G., Fara, A. F., et al. (2013). Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity* 39, 1143–1157. doi: 10.1016/j.immuni.2013.10.018

Liu, J. S., Zhao, M. L., Brosnan, C. F., and Lee, S. C. (2001). Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. *Am. J. Pathol.* 158, 2057–2066. doi: 10.1016/S0002-9440(10)64677-9

Longoni, G., Rocca, M. A., Pagani, E., Riccitelli, G. C., Colombo, B., Rodegher, M., et al. (2015). Deficits in memory and visuospatial learning correlate with regional hippocampal atrophy in MS. *Brain Struct. Funct.* 220, 435–444. doi: 10.1007/s00429-013-0665-9

Loveless, S., Neal, J. W., Howell, O. W., Harding, K. E., Sarkies, P., Evans, R., et al. (2018). Tissue microarray methodology identifies complement pathway activation and dysregulation in progressive multiple sclerosis. *Brain Pathol.* 28, 507–520. doi: 10.1111/bpa.12546

Lublin, F. D., Reingold, S. C., Cohen, J. A., Cutter, G. R., Sorensen, P. S., Thompson, A. J., et al. (2014). Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology* 83, 278–286. doi: 10.1212/WNL.0000000000000560

Lucchinetti, C., Bruck, W., Parisi, J., Scheithauer, B., Rodriguez, M., and Lassmann, H. (2000). Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* 47, 707–717. doi: 10.1002/1531-8249(200006)47:6<707::aid-ana3>3.0.co;2-q

Lucchinetti, C. F., Popescu, B. F., Bunyan, R. F., Moll, N. M., Roemer, S. F., Lassmann, H., et al. (2011). Inflammatory cortical demyelination in early multiple sclerosis. *N. Engl. J. Med.* 365, 2188–2197. doi: 10.1056/NEJMoa1100648

Lui, H., Zhang, J., Makinson, S. R., Cahill, M. K., Kelley, K. W., Huang, H. Y., et al. (2016). Progranulin deficiency promotes circuit-specific synaptic pruning by microglia *via* complement activation. *Cell* 165, 921–935. doi: 10.1016/j.cell.2016.04.001

Lumsden, C. E. (1971). The immunogenesis of the multiple sclerosis plaque. *Brain Res.* 28, 365–390. doi: 10.1016/0006-8993(71)90052-7

Macaron, G., and Ontaneda, D. (2019). Diagnosis and management of progressive multiple sclerosis. *Biomedicines* 7:56. doi: 10.3390/biomedicines7030056

Machado-Santos, J., Saji, E., Troscher, A. R., Paunovic, M., Liblau, R., Gabriely, G., et al. (2018). The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8+ T lymphocytes and B cells. *Brain* 141, 2066–2082. doi: 10.1093/brain/awy151

Magliozzi, R., Hametner, S., Facchiano, F., Marastoni, D., Rossi, S., Castellaro, M., et al. (2019). Iron homeostasis, complement and coagulation cascade as CSF signature of cortical lesions in early multiple sclerosis. *Ann. Clin. Transl. Neurol.* 6, 2150–2163. doi: 10.1002/acn3.50893

Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M., et al. (2007). Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* 130, 1089–1104. doi: 10.1093/brain/awm038

Magliozzi, R., Howell, O. W., Nicholas, R., Cruciani, C., Castellaro, M., Romualdi, C., et al. (2018). Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. *Ann. Neurol.* 83, 739–755. doi: 10.1002/ana.25197

Magliozzi, R., Howell, O. W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., et al. (2010). A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann. Neurol.* 68, 477–493. doi: 10.1002/ana.22230

Mahad, D. J., Ziabreva, I., Campbell, G., Lax, N., White, K., Hanson, P. S., et al. (2009). Mitochondrial changes within axons in multiple sclerosis. *Brain* 132, 1161–1174. doi: 10.1093/brain/awp046

Maier, M., Peng, Y., Jiang, L., Seabrook, T. J., Carroll, M. C., and Lemere, C. A. (2008). Complement C3 deficiency leads to accelerated amyloid β plaque deposition and neurodegeneration and modulation of the microglia/macrophage phenotype in amyloid precursor protein transgenic mice. *J. Neurosci.* 28, 6333–6341. doi: 10.1523/JNEUROSCI.0829-08.2008

Mallucci, G. R. (2009). Prion neurodegeneration: starts and stops at the synapse. *Prion* 3, 195–201. doi: 10.4161/pri.3.4.9981

Marik, C., Felts, P. A., Bauer, J., Lassmann, H., and Smith, K. J. (2007). Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? *Brain* 130, 2800–2815. doi: 10.1093/brain/awm236

Markiewski, M. M., Nilsson, B., Ekdahl, K. N., Mollnes, T. E., and Lambris, J. D. (2007). Complement and coagulation: strangers or partners in crime? *Trends Immunol.* 28, 184–192. doi: 10.1016/j.it.2007.02.006

Martin, M., and Blom, A. M. (2016). Complement in removal of the dead—balancing inflammation. *Immunol. Rev.* 274, 218–232. doi: 10.1111/imr.12462

Martinez-Forero, I., Garcia-Munoz, R., Martinez-Pasamar, S., Inoges, S., Lopez-Diaz de Cerio, A., Palacios, R., et al. (2008). IL-10 suppressor activity and *ex vivo* Tr1 cell function are impaired in multiple sclerosis. *Eur. J. Immunol.* 38, 576–586. doi: 10.1002/eji.200737271

Martinez Sosa, S., and Smith, K. J. (2017). Understanding a role for hypoxia in lesion formation and location in the deep and periventricular white matter in small vessel disease and multiple sclerosis. *Clin. Sci.* 131, 2503–2524. doi: 10.1042/CS20170981

Masuda, T., Amann, L., Sankowski, R., Staszewski, O., Lenz, M., Errico, P. D., et al. (2020). Novel Hexb-based tools for studying microglia in the CNS. *Nat. Immunol.* 21, 802–815. doi: 10.1038/s41590-020-0707-4

Mi, Z. P., Jiang, P., Weng, W. L., Lindberg, F. P., Narayanan, V., and Lagenaur, C. F. (2000). Expression of a synapse-associated membrane protein, P84/SHPS-1 and its ligand, IAP/CD47, in mouse retina. *J. Comp. Neurol.* 416, 335–344. doi: 10.1002/(SICI)1096-9861(20000117)416:33.0.CO;2-X

Michailidou, I., Jongejan, A., Vreijling, J. P., Georgakopoulou, T., de Wissel, M. B., Wolterman, R. A., et al. (2018). Systemic inhibition of the membrane attack complex impedes neuroinflammation in chronic relapsing experimental autoimmune encephalomyelitis. *Acta Neuropathol. Commun.* 6:36. doi: 10.1186/s40478-018-0536-y

Michailidou, I., Naessens, D. M., Hametner, S., Guldenaar, W., Kooi, E. J., Geurts, J. J., et al. (2017). Complement C3 on microglial clusters in multiple sclerosis occur in chronic but not acute disease: implication for disease pathogenesis. *Glia* 65, 264–277. doi: 10.1002/glia.23090

Michailidou, I., Willems, J. G., Kooi, E. J., van Eden, C., Gold, S. M., Geurts, J. J., et al. (2015). Complement C1q-C3-associated synaptic changes in multiple sclerosis hippocampus. *Ann. Neurol.* 77, 1007–1026. doi: 10.1002/ana.24398

Micu, I., Jiang, Q., Coderre, E., Ridsdale, A., Zhang, L., Woulfe, J., et al. (2006). NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. *Nature* 439, 988–992. doi: 10.1038/nature04474

Moller, T. (2010). Neuroinflammation in Huntington's disease. *J. Neural Transm.* 117, 1001–1008. doi: 10.1007/s00702-010-0430-7

Morgan, B. P., and Gasque, P. (1997). Extrahepatic complement biosynthesis: where, when and why? *Clin. Exp. Immunol.* 107, 1–7. doi: 10.1046/j.1365-2249.1997.d01-890.x

Morgan, B. P., and Harris, C. L. (2015). Complement, a target for therapy in inflammatory and degenerative diseases. *Nat. Rev. Drug Discov.* 14, 857–877. doi: 10.1038/nrd4657

Nguyen, H. X., Galvan, M. D., and Anderson, A. J. (2008). Characterization of early and terminal complement proteins associated with polymorphonuclear leukocytes *in vitro* and *in vivo* after spinal cord injury. *J. Neuroinflammation* 5:26. doi: 10.1186/1742-2094-5-26

Nishimoto, H., Yamamoto, A., Furukawa, S., Wakisaka, S., and Maeda, T. (2017). C1q/TNF-related protein 3 expression and effects on adipocyte differentiation of 3T3–L1 cells. *Cell Biol. Int.* 41, 197–203. doi: 10.1002/cbin.10674

Okazawa, H., Motegi, S., Ohyama, N., Ohnishi, H., Tomizawa, T., Kaneko, Y., et al. (2005). Negative regulation of phagocytosis in macrophages by the CD47-SHPS-1 system. *J. Immunol.* 174, 2004–2011. doi: 10.4049/jimmunol.174.4.2004

Oldenborg, P. A., Gresham, H. D., and Lindberg, F. P. (2001). CD47-signal regulatory protein α (SIRPalpha) regulates Fcγ and complement receptor-mediated phagocytosis. *J. Exp. Med.* 193, 855–862. doi: 10.1084/jem.193.7.855

Oldenborg, P. A., Zheleznyak, A., Fang, Y. F., Lagenaur, C. F., Gresham, H. D., and Lindberg, F. P. (2000). Role of CD47 as a marker of self on red blood cells. *Science* 288, 2051–2054. doi: 10.1126/science.288.5473.2051

Papadopoulos, D., Dukes, S., Patel, R., Nicholas, R., Vora, A., and Reynolds, R. (2009). Substantial archaeocortical atrophy and neuronal loss in multiple sclerosis. *Brain Pathol.* 19, 238–253. doi: 10.1111/j.1750-3639.2008.00177.x

Pasinetti, G. M., Johnson, S. A., Rozovsky, I., Lampert-Etchells, M., Morgan, D. G., Gordon, M. N., et al. (1992). Complement C1qB and C4 mRNAs responses to lesioning in rat brain. *Exp. Neurol.* 118, 117–125. doi: 10.1016/0014-4886(92)90028-o

Paterson, P. Y. (1976). Experimental allergic encephalomyelitis: role of fibrin deposition in immunopathogenesis of inflammation in rats. *Fed. Proc.* 35, 2428–2434.

Peterson, J. W., Bo, L., Mork, S., Chang, A., and Trapp, B. D. (2001). Transected neurites, apoptotic neurons and reduced inflammation in cortical multiple sclerosis lesions. *Ann. Neurol.* 50, 389–400. doi: 10.1002/ana.1123

Pikor, N. B., Astarita, J. L., Summers-Deluca, L., Galicia, G., Qu, J., Ward, L. A., et al. (2015). Integration of Th17- and lymphotoxin-derived signals initiates meningeal-resident stromal cell remodeling to propagate neuroinflammation. *Immunity* 43, 1160–1173. doi: 10.1016/j.immuni.2015.11.010

Popescu, B. F., Bunyan, R. F., Parisi, J. E., Ransohoff, R. M., and Lucchinetti, C. F. (2011). A case of multiple sclerosis presenting with inflammatory cortical demyelination. *Neurology* 76, 1705–1710. doi: 10.1212/WNL.0b013e31821a44f1

Prineas, J. W., Kwon, E. E., Cho, E. S., Sharer, L. R., Barnett, M. H., Oleszak, E. L., et al. (2001). Immunopathology of secondary-progressive multiple sclerosis. *Ann. Neurol.* 50, 646–657. doi: 10.1002/ana.1255

Qiao, F., Atkinson, C., Kindy, M. S., Shunmugavel, A., Morgan, B. P., Song, H., et al. (2010). The alternative and terminal pathways of complement mediate post-traumatic spinal cord inflammation and injury. *Am. J. Pathol.* 177, 3061–3070. doi: 10.2353/ajpath.2010.100158

Qiao, F., Atkinson, C., Song, H., Pannu, R., Singh, I., and Tomlinson, S. (2006). Complement plays an important role in spinal cord injury and represents a therapeutic target for improving recovery following trauma. *Am. J. Pathol.* 169, 1039–1047. doi: 10.2353/ajpath.2006.060248

Ramaglia, V., Florescu, A., Zuo, M., Sheikh-Mohamed, S., and Gommerman, J. L. (in press a). Stromal cell-mediated coordination of immune cell recruitment, retention, and function in brain-adjacent regions. *J. Immunol.* 206.

Ramaglia, V., Rojas, O., Naouar, I., and Gommerman, J. L. (in press b). The Ins and outs of central nervous system inflammation—lessons learned from multiple sclerosis. *Annu. Rev. Immunol.* 39.

Ramaglia, V., Hughes, T. R., Donev, R. M., Ruseva, M. M., Wu, X., Huitinga, I., et al. (2012). C3-dependent mechanism of microglial priming relevant to multiple sclerosis. *Proc. Natl. Acad. Sci. U S A* 109, 965–970. doi: 10.1073/pnas.1111924109

Ramaglia, V., Jackson, S. J., Hughes, T. R., Neal, J. W., Baker, D., and Morgan, B. P. (2015). Complement activation and expression during chronic relapsing experimental autoimmune encephalomyelitis in the Biozzi ABH mouse. *Clin. Exp. Immunol.* 180, 432–441. doi: 10.1111/cei.12595

Ramaglia, V., King, R. H., Morgan, B. P., and Baas, F. (2009a). Deficiency of the complement regulator CD59a exacerbates Wallerian degeneration. *Mol. Immunol.* 46, 1892–1896. doi: 10.1016/j.molimm.2009.01.017

Ramaglia, V., King, R. H., Nourallah, M., Wolterman, R., de Jonge, R., Ramkema, M., et al. (2007). The membrane attack complex of the complement system is essential for rapid Wallerian degeneration. *J. Neurosci.* 27, 7663–7672. doi: 10.1523/JNEUROSCI.5623-06.2007

Ramaglia, V., Sheikh-Mohamed, S., Legg, K., Park, C., Rojas, O. L., Zandee, S., et al. (2019). Multiplexed imaging of immune cells in staged multiple sclerosis lesions by mass cytometry. *eLife* 8:e48051. doi: 10.7554/eLife.48051

Ramaglia, V., Tannemaat, M. R., de Kok, M., Wolterman, R., Vigar, M. A., King, R. H., et al. (2009b). Complement inhibition accelerates regeneration in a model of peripheral nerve injury. *Mol. Immunol.* 47, 302–309. doi: 10.1016/j.molimm.2009.09.019

Ramaglia, V., Wolterman, R., de Kok, M., Vigar, M. A., Wagenaar-Bos, I., King, R. H., et al. (2008). Soluble complement receptor 1 protects the peripheral nerve from early axon loss after injury. *Am. J. Pathol.* 172, 1043–1052. doi: 10.2353/ajpath.2008.070660

Reali, C., Magliozzi, R., Roncaroli, F., Nicholas, R., Howell, O. W., and Reynolds, R. (2020). B cell rich meningeal inflammation associates with increased spinal cord pathology in multiple sclerosis. *Brain Pathol.* 30, 779–793. doi: 10.1111/bpa.12841

Reinhold, M. I., Lindberg, F. P., Plas, D., Reynolds, S., Peters, M. G., and Brown, E. J. (1995). *In vivo* expression of alternatively spliced forms of integrin-associated protein (CD47). *J. Cell Sci.* 108, 3419–3425.

Ricklin, D., Hajishengallis, G., Yang, K., and Lambris, J. D. (2010). Complement: a key system for immune surveillance and homeostasis. *Nat. Immunol.* 11, 785–797. doi: 10.1038/ni.1923

Ricklin, D., and Lambris, J. D. (2013). Complement in immune and inflammatory disorders: pathophysiological mechanisms. *J. Immunol.* 190, 3831–3838. doi: 10.4049/jimmunol.1203487

Rindfleisch, E. (1863). Histologisches detail zur grauen degeneration von gehirn und ruckenmark. *Archiv F. Pathol. Anat.* 26, 474–483. doi: 10.1007/BF01878008

Rodriguez, M., and Scheithauer, B. (1994). Ultrastructure of multiple sclerosis. *Ultrastruct. Pathol.* 18, 3–13. doi: 10.3109/01913129409016267

Rua, R., and McGavern, D. B. (2018). Advances in meningeal immunity. *Trends Mol. Med.* 24, 542–559. doi: 10.1016/j.molmed.2018.04.003

Ruseva, M. M., Ramaglia, V., Morgan, B. P., and Harris, C. L. (2015). An anticomplement agent that homes to the damaged brain and promotes recovery after traumatic brain injury in mice. *Proc. Natl. Acad. Sci. U S A* 112, 14319–14324. doi: 10.1073/pnas.1513698112

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705. doi: 10.1016/j.neuron.2012.03.026

Schaffler, A., and Buechler, C. (2012). CTRP family: linking immunity to metabolism. *Trends Endocrinol. Metab.* 23, 194–204. doi: 10.1016/j.tem.2011.12.003

Schneider-Hohendorf, T., Stenner, M. P., Weidenfeller, C., Zozulya, A. L., Simon, O. J., Schwab, N., et al. (2010). Regulatory T cells exhibit enhanced migratory characteristics, a feature impaired in patients with multiple sclerosis. *Eur. J. Immunol.* 40, 3581–3590. doi: 10.1002/eji.201040558

Schneyer, C. A., and Hall, H. D. (1975). Parasympathetic regulation of mitosis induced in rat parotid by dietary change. *Am. J. Physiol.* 229, 1614–1617. doi: 10.1152/ajplegacy.1975.229.6.1614

Schwab, C., and McGeer, P. L. (2002). Complement activated C4d immunoreactive oligodendrocytes delineate small cortical plaques in multiple sclerosis. *Exp. Neurol.* 174, 81–88. doi: 10.1006/exnr.2001.7851

Scott-Hewitt, N., Perrucci, F., Morini, R., Erreni, M., Mahoney, M., Witkowska, A., et al. (2020). Local externalization of phosphatidylserine mediates developmental synaptic pruning by microglia. *EMBO J.* 39:e105380. doi: 10.15252/embj.2020105380

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., and Aloisi, F. (2004). Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 14, 164–174. doi: 10.1111/j.1750-3639.2004.tb00049.x

Sewell, D. L., Nacewicz, B., Liu, F., Macvilay, S., Erdei, A., Lambris, J. D., et al. (2004). Complement C3 and C5 play critical roles in traumatic brain cryoinjury: blocking effects on neutrophil extravasation by C5a receptor antagonist. *J. Neuroimmunol.* 155, 55–63. doi: 10.1016/j.jneuroim.2004.06.003

Sicotte, N. L., Kern, K. C., Giesser, B. S., Arshanapalli, A., Schultz, A., Montag, M., et al. (2008). Regional hippocampal atrophy in multiple sclerosis. *Brain* 131, 1134–1141. doi: 10.1093/brain/awn030

Smith, K. J., Kapoor, R., and Felts, P. A. (1999). Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol.* 9, 69–92. doi: 10.1111/j.1750-3639.1999.tb00212.x

Squire, L. R., Stark, C. E., and Clark, R. E. (2004). The medial temporal lobe. *Annu. Rev. Neurosci.* 27, 279–306. doi: 10.1146/annurev.neuro.27.070203.144130

Steele, M. R., Inman, D. M., Calkins, D. J., Horner, P. J., and Vetter, M. L. (2006). Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. *Invest. Ophthalmol. Vis. Sci.* 47, 977–985. doi: 10.1167/iovs.05-0865

Stephan, A. H., Madison, D. V., Mateos, J. M., Fraser, D. A., Lovelett, E. A., Coutellier, L., et al. (2013). A dramatic increase of C1q protein in the CNS during normal aging. *J. Neurosci.* 33, 13460–13474. doi: 10.1523/JNEUROSCI.1333-13.2013

Stern, J. N., Yaari, G., Vander Heiden, J. A., Church, G., Donahue, W. F., Hintzen, R. Q., et al. (2014). B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes. *Sci. Transl. Med.* 6:248ra107. doi: 10.1126/scitranslmed.3008879

Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., et al. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell* 131, 1164–1178. doi: 10.1016/j.cell.2007.10.036

Stone, L. A., Smith, M. E., Albert, P. S., Bash, C. N., Maloni, H., Frank, J. A., et al. (1995). Blood-brain barrier disruption on contrast-enhanced MRI in patients with mild relapsing-remitting multiple sclerosis: relationship to course, gender and age. *Neurology* 45, 1122–1126. doi: 10.1212/wnl.45.6.1122

Storch, M. K., Piddlesden, S., Haltia, M., Iivanainen, M., Morgan, P., and Lassmann, H. (1998). Multiple sclerosis: *in situ* evidence for antibody- and complement-mediated demyelination. *Ann. Neurol.* 43, 465–471. doi: 10.1002/ana.410430409

Strohmeyer, R., Ramirez, M., Cole, G. J., Mueller, K., and Rogers, J. (2002). Association of factor H of the alternative pathway of complement with agrin and complement receptor 3 in the Alzheimer's disease brain. *J. Neuroimmunol.* 131, 135–146. doi: 10.1016/s0165-5728(02)00272-2

Stys, P. K., You, H., and Zamponi, G. W. (2012a). Copper-dependent regulation of NMDA receptors by cellular prion protein: implications for neurodegenerative disorders. *J. Physiol.* 590, 1357–1368. doi: 10.1113/jphysiol.2011.225276

Stys, P. K., Zamponi, G. W., van Minnen, J., and Geurts, J. J. (2012b). Will the real multiple sclerosis please stand up? *Nat. Rev. Neurosci.* 13, 507–514. doi: 10.1038/nrn3275

Svensson, M., and Aldskogius, H. (1992). Evidence for activation of the complement cascade in the hypoglossal nucleus following peripheral nerve injury. *J. Neuroimmunol.* 40, 99–109. doi: 10.1016/0165-5728(92)90217-9

Tei, R., Kaido, T., Nakase, H., and Sakaki, T. (2008). Protective effect of C1 esterase inhibitor on acute traumatic spinal cord injury in the rat. *Neurol. Res.* 30, 761–767. doi: 10.1179/174313208X284241

Ten, V. S., Yao, J., Ratner, V., Sosunov, S., Fraser, D. A., Botto, M., et al. (2010). Complement component c1q mediates mitochondria-driven oxidative stress in neonatal hypoxic-ischemic brain injury. *J. Neurosci.* 30, 2077–2087. doi: 10.1523/JNEUROSCI.5249-09.2010

Tenner, A. J. (2020). Complement-mediated events in Alzheimer's disease: mechanisms and potential therapeutic targets. *J. Immunol.* 204, 306–315. doi: 10.4049/jimmunol.1901068

Thomas, A., Gasque, P., Vaudry, D., Gonzalez, B., and Fontaine, M. (2000). Expression of a complete and functional complement system by human neuronal cells *in vitro*. *Int. Immunol.* 12, 1015–1023. doi: 10.1093/intimm/12.7.1015

Thomas, W. S., Mori, E., Copeland, B. R., Yu, J. Q., Morrissey, J. H., and del Zoppo, G. J. (1993). Tissue factor contributes to microvascular defects after focal cerebral ischemia. *Stroke* 24, 847–853; discussion 847. doi: 10.1161/01.str.24.6.847

Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E., et al. (2002). Long-term *in vivo* imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 420, 788–794. doi: 10.1038/nature01273

Trapp, B. D., Peterson, J., Ransohoff, R. M., Rudick, R., Mork, S., and Bo, L. (1998). Axonal transection in the lesions of multiple sclerosis. *N. Engl. J. Med.* 338, 278–285. doi: 10.1056/NEJM199801293380502

Trapp, B. D., and Stys, P. K. (2009). Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet Neurol.* 8, 280–291. doi: 10.1016/S1474-4422(09)70043-2

Trapp, B. D., Wujek, J. R., Criste, G. A., Jalabi, W., Yin, X., Kidd, G. J., et al. (2007). Evidence for synaptic stripping by cortical microglia. *Glia* 55, 360–368. doi: 10.1002/glia.20462

Tropea, D., Majewska, A. K., Garcia, R., and Sur, M. (2010). Structural dynamics of synapses *in vivo* correlate with functional changes during experience-dependent plasticity in visual cortex. *J. Neurosci.* 30, 11086–11095. doi: 10.1523/JNEUROSCI.1661-10.2010

Trouw, L. A., Nielsen, H. M., Minthon, L., Londos, E., Landberg, G., Veerhuis, R., et al. (2008). C4b-binding protein in Alzheimer's disease: binding to Aβ1–42 and to dead cells. *Mol. Immunol.* 45, 3649–3660. doi: 10.1016/j.molimm.2008.04.025

Vainchtein, I. D., Chin, G., Cho, F. S., Kelley, K. W., Miller, J. G., Chien, E. C., et al. (2018). Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. *Science* 359, 1269–1273. doi: 10.1126/science.aal3589

van Horssen, J., Brink, B. P., de Vries, H. E., van der Valk, P., and Bo, L. (2007). The blood-brain barrier in cortical multiple sclerosis lesions. *J. Neuropathol. Exp. Neurol.* 66, 321–328. doi: 10.1097/nen.0b013e318040b2de

Vasek, M. J., Garber, C., Dorsey, D., Durrant, D. M., Bollman, B., Soung, A., et al. (2016). A complement-microglial axis drives synapse loss during virus-induced memory impairment. *Nature* 534, 538–543. doi: 10.1038/nature18283

Veerhuis, R. (2011). Histological and direct evidence for the role of complement in the neuroinflammation of AD. *Curr. Alzheimer Res.* 8, 34–58. doi: 10.2174/156720511794604589

Veerhuis, R., Nielsen, H. M., and Tenner, A. J. (2011). Complement in the brain. *Mol. Immunol.* 48, 1592–1603. doi: 10.1016/j.molimm.2011.04.003

Venken, K., Hellings, N., Thewissen, M., Somers, V., Hensen, K., Rummens, J. L., et al. (2008). Compromised CD4+ CD25(high) regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. *Immunology* 123, 79–89. doi: 10.1111/j.1365-2567.2007.02690.x

Viglietta, V., Baecher-Allan, C., Weiner, H. L., and Hafler, D. A. (2004). Loss of functional suppression by $CD4^{+}CD25^{+}$ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 199, 971–979. doi: 10.1084/jem.20031579

Vladimirova, O., O'Connor, J., Cahill, A., Alder, H., Butunoi, C., and Kalman, B. (1998). Oxidative damage to DNA in plaques of MS brains. *Mult. Scler.* 4, 413–418. doi: 10.1177/135245859800400503

Walker, D. G., Kim, S. U., and McGeer, P. L. (1995). Complement and cytokine gene expression in cultured microglial derived from postmortem human brains. *J. Neurosci. Res.* 40, 478–493. doi: 10.1002/jnr.490400407

Wang, C., Yue, H., Hu, Z., Shen, Y., Ma, J., Li, J., et al. (2020). Microglia mediate forgetting *via* complement-dependent synaptic elimination. *Science* 367, 688–694. doi: 10.1126/science.aaz2288

Ward, L. A., Lee, D. S., Sharma, A., Wang, A., Naouar, I., Ma, X. I., et al. (2020). Siponimod therapy implicates Th17 cells in a preclinical model of subpial cortical injury. *JCI Insight* 5:e132522. doi: 10.1172/jci.insight.132522

Watkins, L. M., Neal, J. W., Loveless, S., Michailidou, I., Ramaglia, V., Rees, M. I., et al. (2016). Complement is activated in progressive multiple sclerosis cortical grey matter lesions. *J. Neuroinflammation* 13:161. doi: 10.1186/s12974-016-0611-x

Wegner, C., Esiri, M. M., Chance, S. A., Palace, J., and Matthews, P. M. (2006). Neocortical neuronal, synaptic and glial loss in multiple sclerosis. *Neurology* 67, 960–967. doi: 10.1212/01.wnl.0000237551.26858.39

Werneburg, S., Jung, J., Kunjamma, R. B., Ha, S. K., Luciano, N. J., Willis, C. M., et al. (2020). Targeted complement inhibition at synapses prevents microglial synaptic engulfment and synapse loss in Demyelinating disease. *Immunity* 52, 167.e7–182.e7.doi: 10.1016/j.immuni.2019.12.004

Williams, P. A., Tribble, J. R., Pepper, K. W., Cross, S. D., Morgan, B. P., Morgan, J. E., et al. (2016). Inhibition of the classical pathway of the complement cascade prevents early dendritic and synaptic degeneration in glaucoma. *Mol. Neurodegener.* 11:26. doi: 10.1186/s13024-016-0091-6

Willis, C. M., Nicaise, A. M., Menoret, A., Ryu, J. K., Mendiola, A. S., Jellison, E. R., et al. (2019). Extracellular vesicle fibrinogen induces encephalitogenic $CD8^{+}$ T cells in a mouse model of multiple sclerosis. *Proc. Natl. Acad. Sci. U S A* 116, 10488–10493. doi: 10.1073/pnas.1816911116

Witte, M. E., Bo, L., Rodenburg, R. J., Belien, J. A., Musters, R., Hazes, T., et al. (2009). Enhanced number and activity of mitochondria in multiple sclerosis lesions. *J. Pathol.* 219, 193–204. doi: 10.1002/path.2582

Wyss-Coray, T., Yan, F., Lin, A. H., Lambris, J. D., Alexander, J. J., Quigg, R. J., et al. (2002). Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc. Natl. Acad. Sci. U S A* 99, 10837–10842. doi: 10.1073/pnas.162350199

Yang, Y., Tian, S. J., Wu, L., Huang, D. H., and Wu, W. P. (2011). Fibrinogen depleting agent batroxobin has a beneficial effect on experimental autoimmune encephalomyelitis. *Cell. Mol. Neurobiol.* 31, 437–448. doi: 10.1007/s10571-010-9637-2

You, H., Tsutsui, S., Hameed, S., Kannanayakal, T. J., Chen, L., Xia, P., et al. (2012). Abeta neurotoxicity depends on interactions between copper ions, prion protein and N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. U S A* 109, 1737–1742. doi: 10.1073/pnas.1110789109

Zeis, T., Probst, A., Steck, A. J., Stadelmann, C., Bruck, W., and Schaeren-Wiemers, N. (2009). Molecular changes in white matter adjacent to an active demyelinating lesion in early multiple sclerosis. *Brain Pathol.* 19, 459–466. doi: 10.1111/j.1750-3639.2008.00231.x

Zelek, W. M., Fathalla, D., Morgan, A., Touchard, S., Loveless, S., Tallantyre, E., et al. (2019). Cerebrospinal fluid complement system biomarkers in demyelinating disease. *Mult. Scler.* 26, 1929–1937. doi: 10.1177/1352458519887905

Zrzavy, T., Hametner, S., Wimmer, I., Butovsky, O., Weiner, H. L., and Lassmann, H. (2017). Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* 140, 1900–1913. doi: 10.1093/brain/awx113

Zurawski, J., Tauhid, S., Chu, R., Khalid, F., Healy, B. C., Weiner, H. L., et al. (2020). 7T MRI cerebral leptomeningeal enhancement is common in relapsing-remitting multiple sclerosis and is associated with cortical and thalamic lesions. *Mult. Scler.* 26, 177–187. doi: 10.1177/1352458519885106

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Engine Failure in Axo-Myelinic Signaling: A Potential Key Player in the Pathogenesis of Multiple Sclerosis

*Talia Bergaglio, Antonio Luchicchi and Geert J. Schenk**

Department of Anatomy and Neurosciences, Amsterdam Neuroscience, Amsterdam University Medical Center, Amsterdam MS Center, Amsterdam, Netherlands

Multiple Sclerosis (MS) is a complex and chronic disease of the central nervous system (CNS), characterized by both degenerative and inflammatory processes leading to axonal damage, demyelination, and neuronal loss. In the last decade, the traditional *outside-in* standpoint on MS pathogenesis, which identifies a primary autoimmune inflammatory etiology, has been challenged by a complementary *inside-out* theory. By focusing on the degenerative processes of MS, the axo-myelinic system may reveal new insights into the disease triggering mechanisms. Oxidative stress (OS) has been widely described as one of the means driving tissue injury in neurodegenerative disorders, including MS. Axonal mitochondria constitute the main energy source for electrically active axons and neurons and are largely vulnerable to oxidative injury. Consequently, axonal mitochondrial dysfunction might impair efficient axo-glial communication, which could, in turn, affect axonal integrity and the maintenance of axonal, neuronal, and synaptic signaling. In this review article, we argue that OS-derived mitochondrial impairment may underline the dysfunctional relationship between axons and their supportive glia cells, specifically oligodendrocytes and that this mechanism is implicated in the development of a primary cytodegeneration and a secondary pro-inflammatory response (*inside-out*), which in turn, together with a variably primed host's immune system, may lead to the onset of MS and its different subtypes.

Keywords: oxidative stress, mitochondria, axo-myelinic synapse, multiple sclerosis, neurodegeneration

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****Correspondence:***
Geert J. Schenk
g.schenk@amsterdamumc.nl

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INTRODUCTION

Multiple Sclerosis (MS) is a complex, chronic progressive disorder of the central nervous system (CNS) and the most prominent cause of neurological disability in young adults (Noseworthy et al., 2000; Compston and Coles, 2002; Stys et al., 2012). Comprising of damage to both white and gray matter regions of the brain, evidence from histopathological findings identifies demyelination and axonal damage, as well as microglia activation, synaptic and neuronal loss as hallmarks of the disease (Geurts and Barkhof, 2008; Brinar and Braun, 2013; Klaver et al., 2013; Calabrese et al., 2015). Conflicting ideas have been proposed to explain the etiology of MS, particularly the origin of lesion formation and disease progression. The indisputable involvement of inflammatory processes has resulted in the development of the main model of MS,

namely *outside-in*, whereby a dysregulated immune system in the periphery attacks elements within the CNS, causing demyelination and tissue damage (Stys et al., 2012; Dendrou et al., 2015). Once inflammation becomes compartmentalized within the CNS and compensatory mechanisms can no longer overcome the chronic inflammatory processes, the progressive phenotype takes over and current anti-inflammatory therapeutic strategies are no longer effective (Lassmann et al., 2012). Alternatively, MS may originate from the "*inside-out*," with a primary degenerative episode, possibly evolving around the axo-myelinic synapse (AMS), disrupting the dynamic communication between axons and their myelinating oligodendrocyte, thus initiating a secondary inflammatory response due to the highly antigenic debris derived from myelin breakdown (Trapp and Nave, 2008; Stys et al., 2012). Consequently, the strength of the convolution between degenerative and inflammatory processes will derive the clinical course of the disease.

According to the *inside-out* model, more focus should be aimed at understanding the mechanisms underlying the neurodegenerative processes of MS. Observations of early-stage lesion formation, in both experimental models and human MS, corroborate on the presence of axonal injury in still myelinated axons, suggesting that, at least in some cases, degenerative CNS pathology precedes the peripheral inflammatory attack (Brück and Stadelmann, 2003; Barnett et al., 2009; Edgar et al., 2010; Nikić et al., 2011; Lubetzki and Stankoff, 2014). A potential mechanism driving *inside-out* immunopathogenesis may evolve around the myelinating unit (Stys, 2013). Here, earlier studies in mice revealed a crucial role of myelin molecules and oligodendroglial support in the maintenance of axonal integrity (Griffiths et al., 1998; Yin et al., 1998; Lappe-Siefke et al., 2003). More specifically, axonal metabolic support requires specific axo-myelinic communication, and disruptions to this relationship may induce primary axonal injury and potentially long-lasting myelin abnormalities (Micu et al., 2016).

Axonal and oligodendroglial processes are highly energy-consuming and as such, are vulnerable to metabolic challenges (Micu et al., 2018). Due to the crucial functions mitochondria play as the power plants of a cell, it is not surprising that both genetic and environmental factors altering their functions have profound downstream effects on axonal health (Campbell et al., 2014; Witte et al., 2014). One of the mechanisms that cause injury to mitochondria is Oxidative stress (OS), defined as an imbalance in Reactive Oxygen Species (ROS) production against the cell's antioxidant defenses (Betteridge, 2000). Despite the established involvement of ROS-mediated tissue injury in MS inflammatory processes, the induction of pro-apoptotic mechanisms by OS as well as its impact on the mitochondrial respiratory chain may induce a state of energy deprivation that, if chronic, may ultimately initiate a cascade of degenerative processes, including axonal and neuronal death (Lassmann and van Horssen, 2011; Franklin et al., 2012; Stys, 2013; Ohl et al., 2016).

This mini review article aims to highlight a specific component of MS pathogenesis by lending credence to the role of OS-derived mitochondrial dysfunction as a potential key mechanism contributing to an unstable AMS, which may ultimately contribute to a primary cytodegeneration in MS pathogenesis. To do so, evidence supporting an *inside-out* view of MS will be presented, together with the recent insights into the function of axo-myelinic neurotransmission and the role of axonal mitochondria and OS-associated mitochondrial dysfunction. Here, we propose that OS-derived mitochondrial impairment may underline the dysfunctional relationship between axons and their supportive glia cells, which later initiates the primary cytodegeneration and secondary inflammatory processes of MS.

MULTIPLE SCLEROSIS AS A PRIMARY CYTODEGENERATIVE DISEASE

Although an *outside-in* view of MS pathogenesis cannot be disregarded, the *inside-out* model is equally plausible and equally consistent with the majority of pathological observations. When viewing MS pathogenesis from the *inside-out*, axonal injury and myelin defects likely act as the initiators of the degenerative processes underlying the disease, due to their observation even in the absence of inflammation in human brain samples (Trapp et al., 1998; Traka et al., 2016). Histopathological analysis of patient material from very early stages of the disease has revealed myelin abnormalities, specifically involving the inner myelin sheath and oligodendrocyte loss with little peripheral inflammatory infiltration but with a more pronounced innate immune response (Rodriguez and Scheithauer, 1994; Aboul-Enein et al., 2003; Barnett and Prineas, 2004; Henderson et al., 2009). Moreover, the ineffectiveness of available anti-inflammatory treatments in halting disease progression further exacerbates the presence of underlying cytodegenerative processes driving MS progression (Seewann et al., 2009; Hawker, 2011; Lassmann, 2013). This evidence is corroborated by genetic studies. Immunologically relevant genes are significantly overrepresented in genome-wide association studies (Compston and Coles, 2002). Sawcer et al. (2011) found a strong correlation with immune-related genes within patients with RRMS mainly, which is to be expected due to the great inflammatory character of this MS variant and may explain symptomatic heterogeneity due to a variably primed host's immune system (Stys et al., 2012). Interestingly, however, when a subgroup analysis of only PPMS patients was performed, no associations with genes related to the immune system were found, further indicating that these immune-related factors may only determine the intensity of autoimmune response to a degenerative brain (Stys and Tsutsui, 2019). Instead, a general state of chronic excitotoxicity seems to, at least in part, drive the degenerative processes of MS, whereby variations in genes related to glutamate signaling prevailed in PPMS patients (Baranzini et al., 2010; Strijbis et al., 2013). Due to the chronic and progressive fate of MS, which will rarely be fatal in the early stages, all human neuropathological studies will inevitably mirror a combination of degenerative processes and inflammatory reactions that have evolved over many months or years (Stys, 2013). Hence, it is crucial to critically acknowledge that a single histopathological snapshot in time may not be fully representative of the initial events of MS pathogenesis.

If we consider progressive MS to reflect the real pathogenic mechanisms of the disease, then the origin of lesion formation lays within episodes of axonal injury and disruptions to axo-myelinic communication (Lassmann et al., 2012; Friese et al., 2014; Mahad et al., 2015; Guttmann et al., 2016). Thus, axonal injury may start at an early, and yet not observable, stage of the disease and it does not initially manifest in neurological disability (Trapp et al., 1998). Indeed, the CNS comprises many reparative mechanisms that allow for the compensation of axonal loss (Bjartmar et al., 2003). Once acute demyelination progresses into a more chronic state, demyelinated axons hardly survive and degenerative mechanisms become more prevalent. Approximately up to 60–70% of axonal loss was estimated in chronic white matter lesions in severely disabled MS patients (Mews et al., 1998). It is still hypothetical which exact mechanism may generate damage to the axons and whether axonal injury may represent a primary degenerative process, or maybe caused by secondary, non-inflammatory, processes (Stys et al., 2012). However, disruption of the close dynamic relationship between axons and their insulating myelin sheaths has been identified as a potential mode of action leading to a state of energy deficiency and, consequently, axonal injury (Tsutsui and Stys, 2013; Simons et al., 2014).

AXON-GLIA INTERACTION

The architecture of the axo-myelinic unit is very intricate. Although the myelin sheath supports the electrical properties of the axon, it simultaneously limits the access of the axon to its extracellular environment (Nave, 2010; Simons et al., 2014). Nevertheless, the studies cited provide strong evidence for the implication of the myelinating oligodendrocytes in sustaining the axons by providing the necessary metabolic support (Stys, 2011; Fünfschilling et al., 2012; Lee et al., 2012; Micu et al., 2016). The large and complex crosstalk network between axons and oligodendrocytes constitutes an essential part of the proper functioning of the CNS and exposes the system to a diffuse vulnerability to disorders affecting the myelin (Ortiz et al., 2016).

Despite the close relationship between axons and myelin, only recently, an activity-dependent communication between the two was proposed, namely the axo-myelinic synapse, whose arrangement presents very similar features to the traditional interneuronal synapses (Stys, 2011; Micu et al., 2016, 2018). One critical function of the AMS is thus to couple electrical activity along the axons to the metabolic output from the oligodendrocytes. Remarkably, axons may signal the oligodendrocyte, through AMPA and NMDA myelinic receptors, to supply certain metabolites to fuel the electrically active fiber in response to physiological stimuli; as a result, the glial cells will transfer such metabolites to the internodal axon, providing it with the necessary energy support for signal conductance (Stys, 2011; Micu et al., 2018). In this view, Micu et al. (2016, 2018) propose a potential mechanism of neurotransmission underlying the tightly orchestrated complex directed by the AMS, which is further described in **Figure 1**.

The continuous supply of energy along the entire length of the myelinated axon is crucial for the efficient conduction of action potentials as well as for the proper maintenance of neuronal functioning, including its myelinating system. Consequently, the energy supply from the myelinating oligodendrocytes is suggested to be of vital importance to fuelling the axon, following transient increases in energy demands and, more generally, to the vast dynamic range of firing frequencies of myelinated axons (Trevisiol et al., 2017). Glucose is the primary energy source in the adult brain and it fuels neuronal activity *via* aerobic respiration by mitochondria (Su et al., 2009). Given the crucial role of mitochondria in driving the majority of cellular processes by providing the necessary energy, it is not surprising that mitochondrial dysfunction can result in significant neuronal injury and degenerative processes (Lin and Beal, 2006; Mahad et al., 2008; Witte et al., 2010).

The balance between ROS production and antioxidant defenses under normal physiological conditions can be disrupted with an overproduction of free radicals, namely OS. Oxidants can play a dual role as both toxic and beneficial compounds, the latter due to their crucial role as essential signaling molecules (Pham-Huy et al., 2008). They are produced from both endogenous (e.g., cell metabolism) and exogenous (e.g., cigarette smoking, environmental toxins, chronic physiological stress, iron overload, inflammation, et cetera) sources and can contribute to disease *via* disruptions to cellular homeostasis by redox signaling (Bhattacharyya et al., 2014; Basria et al., 2019). Over time, exposure to multiple inciting factors, as well as the failure of enzymes responsible for redox homeostasis and detoxification activities, may trigger a chronic imbalance between ROS production and antioxidant defenses (Bhattacharyya et al., 2014; Basria et al., 2019). Excess free radicals can lead to mitochondrial dysfunction by inducing mitochondrial DNA mutations, damage to its respiratory chain, alteration of its membrane permeability, and by influencing Ca^{2+} homeostasis and mitochondrial defense systems (Guo et al., 2013). Once damaged, mitochondrial dysfunctional processes can further amplify OS and generate tissue injury *via* three crucial mechanisms, including energy failure, induction of apoptosis, and enhanced production of ROS (Witte et al., 2014). Energy deficiency derived from mitochondrial dysfunction poses the axon to a state of 'virtual hypoxia', whereby axon-glia energy metabolism would directly influence axon-myelin transmission and result in chronic and progressive damage to the axons (Lassmann et al., 2012). More specifically, when energy failure occurs, sodium ions begin to accumulate within the axon, causing, together with membrane depolarization, the reverse mode of action of the Na^{+}-Ca^{2+} exchanger (Franklin et al., 2012; Campbell et al., 2014). As a result, detrimental levels of calcium ions build up to a point where they can interfere with axon survival and lead to axonal injury (Tsutsui and Stys, 2013). Through *in vivo* calcium imaging, Witte et al. (2019) have shown that activation of calpains, Ca^{2+}-dependent, non-lysosomal proteases, upon increased levels of axoplasmic Ca^{2+}, can promote the breakdown of the cytoskeleton as well as oncotic axonal swelling. Activated calpains are also responsible for the permeabilization of lysosomal membranes, which in turn causes the release of the lysosomal hydrolytic enzyme Cathepsin, an important mediator of apoptosis (Czaja, 2001). Both activation of Ca^{2+}-dependent proteases, as well

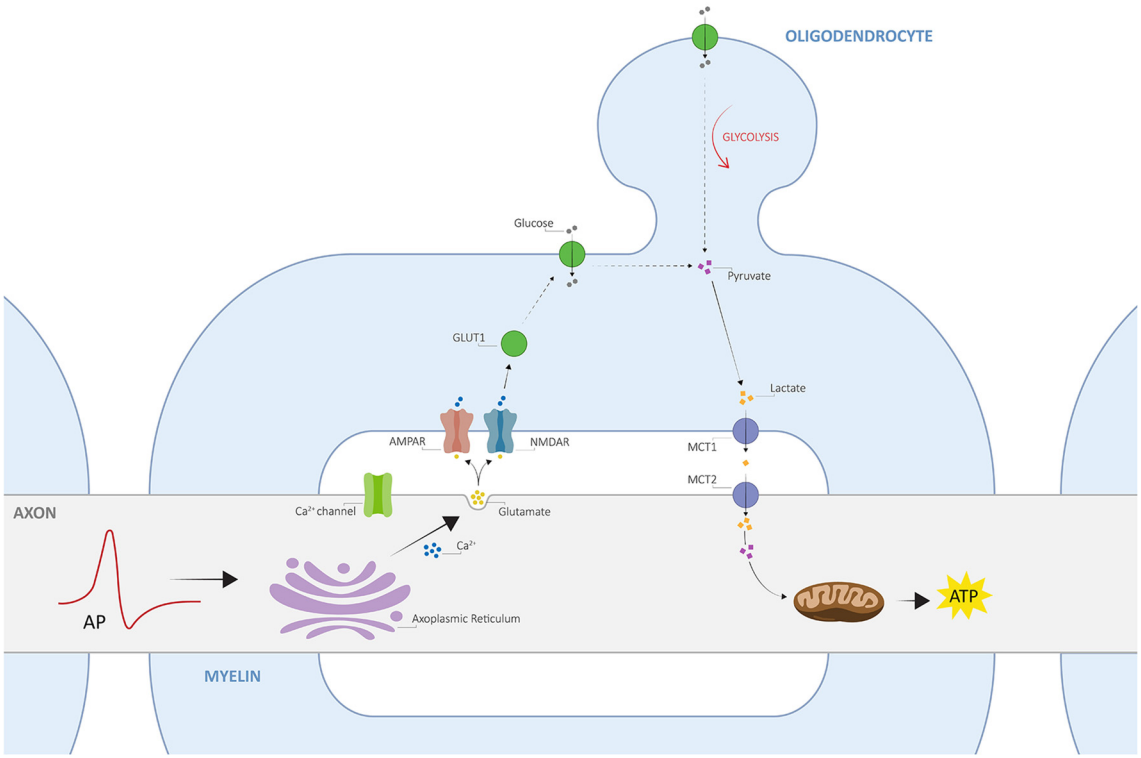

FIGURE 1 | The architecture of axo-myelinic neurotransmission. Traversing action potentials cause depolarization of the axon. The latter is sensed by voltage-gated Ca^{2+} channels, which in turn activate and cause intra-axonal Ca^{2+} release from the axoplasmic reticulum. This stimulates the fusion of glutamatergic vesicles and the consequent release of glutamate into the periaxonal space, which in turn activates myelinic AMPA and NMDA receptors, located on the innermost myelin sheaths (AMPARs and NMDARs, respectively), finally promoting Ca^{2+} influx into the myelin cytoplasm. Myelin receptor activation further results in the recruitment of glucose transporter type 1 (GLUT1), increased uptake of glucose, and the stimulation of glycolysis by the oligodendrocyte, where the production of pyruvate and lactate is enhanced. Pyruvate is then used as an energy supply by myelinic mitochondria, whereas lactate is transported across the periaxonal space and into the axon by monocarboxylate transporters 1 and 2 (MCT1 and MCT2). Finally, lactate is used to fuel aerobic metabolism by axonal mitochondria for the efficient internodal production of ATP. Adapted from Micu et al. (2018), with permission from the authors and SpringerNature.

as mitochondrial impairment, have been associated with the degradation of myelin-associated glycoprotein (MAG), an adhesive protein located on the periaxonal surface of myelin sheaths and responsible for the inhibition of axon regeneration and the control of myelin formation and maintenance (Kroemer and Jäättelä, 2005; Zong and Thompson, 2006). MAG loss, together with other myelinic proteins, is thought to destabilize the AMS by dysregulating the axon cytoskeleton and by inducing myelin breakdown, further exacerbating myelin-axon communication (Schnaar and Lopez, 2009).

MITOCHONDRIAL OXIDATIVE STRESS IN AXO-MYELINIC NEUROTRANSMISSION: A KEY PLAYER IN MS PRIMARY DEGENERATION?

Here, we propose an alternative view of MS pathogenesis, whereby OS-derived mitochondrial impairment acts as the key initiator of a disrupted axo-myelinic relationship and consequently drives the onset of primary cytodegenerative and secondary inflammatory processes (**Figure 2**).

Dysfunctional activation of axo-myelinic neurotransmission may be driven by complementary processes originating from excess free radical production. OS can directly affect mitochondrial functioning *via* different pathways, each leading to a state of energy failure within the affected axons (Guo et al., 2013). The presence of excess ROS, including hydrogen peroxide, puts pressure on cellular antioxidant defense systems, such as glutathione peroxidase (GPx), to restore metabolic balance (Carvalho et al., 2014). GPx is an intracellular antioxidant enzyme that reduces hydrogen peroxide to water to both limit its harmful effects and, indirectly, to modulate mitochondrial oxidative phosphorylation (Lubos et al., 2011). Consequently, with increasing levels of oxyradicals, the production of ATP by axonal mitochondria can be depleted *via* the GPx system. Additionally, the accumulation of calcium ions derived from OS processes would result in detrimental effects not only on the axon itself, *via* over-activation of Ca^{2+}-dependent axonal enzymes, but also on the overlying myelin sheath, by stimulating excessive vesicular glutamate release into the periaxonal

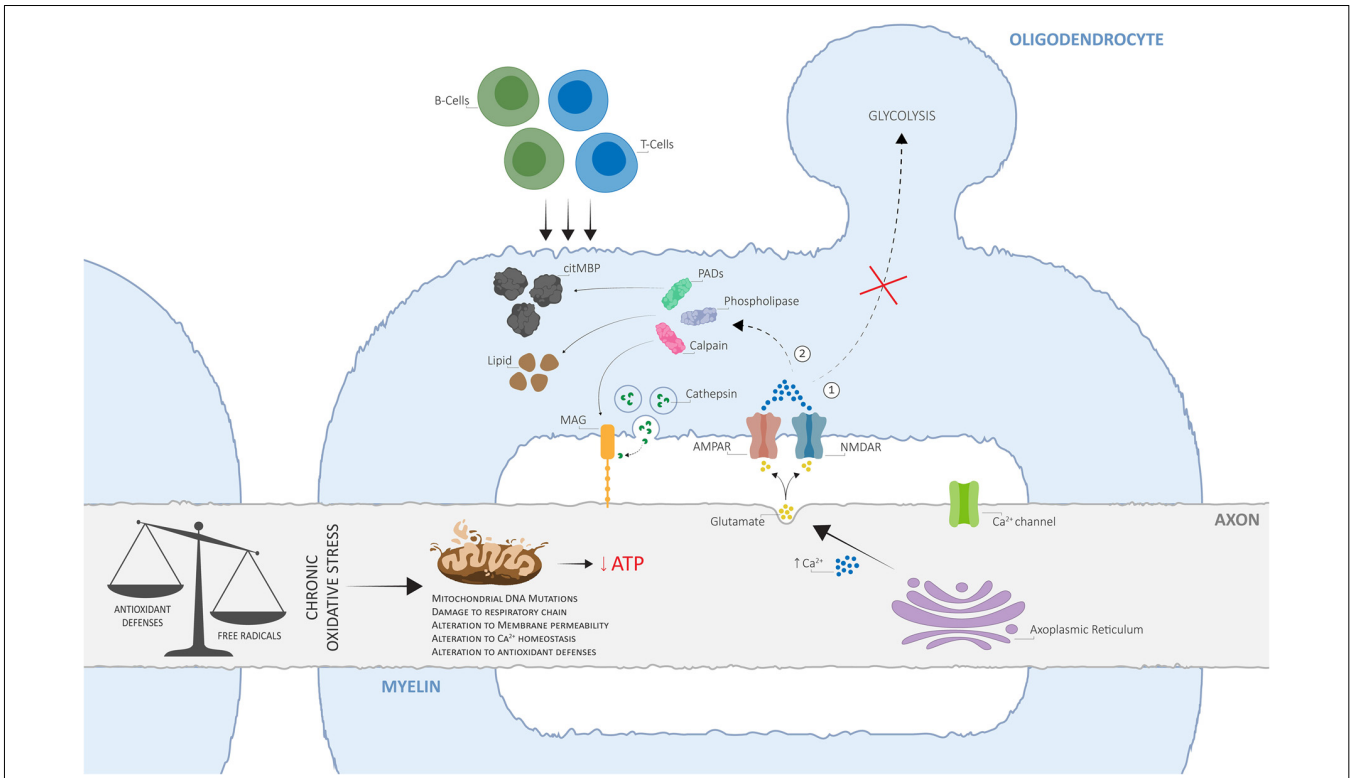

FIGURE 2 | The alternative view of Multiple Sclerosis (MS) pathogenesis is based on dysfunctional axo-myelinic neurotransmission due to oxidative stress (OS)-derived mitochondrial impairment. Chronic OS mechanisms, originating from excess free radical production (due to cell metabolism, physiological stress, iron overload, environmental toxins, cigarette smoking, et cetera) in relation to the cell's antioxidant defenses, can result in the inability of the axonal mitochondria to synthesize ATP, leading to a state of chronic virtual hypoxia (energy failure). As a result, the failure of ion transporters and the consequent influx of Na^+ ions, activate voltage-gated Ca^{2+} channels and induce the excessive intra-axonal release of Ca^{2+}. The latter stimulates the excessive vesicular release of glutamate into the periaxonal space, over-activating myelinic AMPA and NMDA receptors and resulting in excessive Ca^{2+} influx into the myelin cytoplasm, leading to two crucial pathological outcomes: **(1)** the inability of the oligodendrocyte to generate pyruvate and lactate, by glycolysis, for metabolic support to the axon; **(2)** activation of Ca^{2+}-dependent calpains, phospholipases, and PADs, resulting in the degradation of myelin proteins, including myelin-associated glycoprotein (MAG), phospholipids, and in the conversion of MBP into citMBP, respectively, thus inducing focal disruptions to the myelin sheath and destabilization of the axo-myelinic synapse (AMS). The consequent release of antigenic citMBP and lipid debris can result in an adaptive immune response, driven by T- and B-cells, which can cause further inflammatory reactions. Adapted from Micu et al. (2018), with permission from the authors and SpringerNature.

space. Elevated Ca^{2+} levels also inhibit axonal transport of mitochondria, thus immobilizing stationary mitochondria to the affected sites, leading to the degeneration of the entire axon and, consequently, neuronal injury (Su et al., 2009). Most importantly, over time, excessive Ca^{2+} entry into the myelin sheath, together with a state of energy deprivation, will cause both the myelin and the oligodendrocyte to become unable to buffer the Ca^{2+} loads, thus hindering the transport of lactate to the axon for metabolic support (Tsutsui and Stys, 2013; Micu et al., 2018; Poerwoatmodjo et al., 2020). A recent study presented evidence for impaired glycolysis and mitochondrial respiration during T-cell activation in RRMS patients (La Rocca et al., 2017). Here, these changes were associated with a down-regulation of GLUT1 resulting from the enhanced entry of calcium ions inside the myelin sheath. Also, the inability to buffer elevated Ca^{2+} levels will likely trigger a series of enzymatic pathways leading to the degradation of myelin proteins and phospholipids and thus, to axonal demyelination (Stys, 2013; Micu et al., 2018). Finally, the loss of positive charge on citrullinated myelin basic protein (citMBP), induced by the conversion of positively charged arginine residues on MBP to citrulline by peptidyl arginine deiminases (Ca^{2+}-dependent enzymes; PADs), causes focal disruption to the myelin sheath (Caprariello et al., 2018; Micu et al., 2018). The consequent release of antigenic citMBP and lipid debris can result in an adaptive immune response in a host with a reactive immune system (Micu et al., 2018). Primary injury to axonal mitochondria and subsequent demyelination may, in turn, attract leukocytes from the bloodstream, which is in line with the observation that lesions and cell infiltration tend to occur around blood vessels (Gaitán et al., 2013; Lopes Pinheiro et al., 2016). This autoimmune attack, driven by peripheral T- and B-cells, resembles the primary immune-mediated inflammatory response widely described by supporters of the *outside-in* model of MS. Therefore, although the loss of metabolic support may be the initiator of progressive degeneration in MS, it is unlikely to be the only underlying mechanism; a gain of toxic function is likely to promote

further, and potentially irreversible, neurological damage (Micu et al., 2018).

Given the supportive interdependence of neuronal and glial health, pathology from a single axon may spread throughout the nervous system following both a transversal pattern, as well as a process similar to Wallerian degeneration in the peripheral nervous system (PNS; Chong et al., 2012; Singh et al., 2013; Simons et al., 2014). Both spreading modalities can ultimately reflect the pattern of pathology in MS patients, whereby both a longitudinal spread across the pyramidal tracts and a more transversal spread in white and gray matter regions of the CNS can be observed (DeLuca et al., 2004). Given the comparable metabolic rate of white and gray matter, both regions are equally highly vulnerable to interruptions of energy supply (Goldberg and Ransom, 2003). Depending on the location of the primary insult, differences in clinical outcomes, as well as the extent of neurological disability, may vary.

Although the increased energy demand of an axon may not be sufficient to trigger axonal degeneration, energy deprivation likely renders neurons more vulnerable to stress (Simons et al., 2014). Moreover, age-related iron accumulation in the human brain, largely stored within the myelin sheaths, may further amplify oxidative injury to the axo-myelinic unit when it is liberated upon demyelination and may be partly responsible for the presence of activated and reactive microglia and macrophages in MS lesions (Lassmann and van Horssen, 2011; Haider, 2015). Given that processes of cytodegeneration, including early mitochondrial dysfunction associated with OS, occur during the first stages of MS, even when no apparent clinical symptom is visible, it is fair to assume that cellular antioxidant defense mechanisms may be capable of controlling the extent of oxidative damage (Fischer et al., 2012). Additionally, efficient remyelinating mechanisms may be able to sustain the effects of the oxidative injury on axonal degeneration in the early stages of the disease (Franklin and ffrench-Constant, 2008). The severity of the imbalance between OS and antioxidant defenses may contribute to the severity of MS pathology and ultimately, to neurological disability.

DISCUSSION

To elucidate whether cytodegeneration or an autoimmune attack may represent the initial trigger of MS, we presented an alternative view on MS pathogenesis, which identifies chronic OS-derived mitochondrial dysfunction as the main initiator of a primary cytodegeneration and secondary inflammatory response. Here, we hypothesized that mitochondrial-derived energy failure caused by oxidative injury represents a potential mechanism by which the AMS might contribute to MS pathogenesis. The mitochondria can be thought of as a "car engine," which burns fuel and recovers the energy to drive cellular processes. When dysfunctional, however, the engine not only will not be as efficient in producing energy, but it will also create toxic by-products as a result of defective combustion. This toxic debris, generated by the degradation of myelin components as well as by the release of biochemical elements from the dysfunctional mitochondria, may elicit a secondary, inflammatory response, generating a cascade of detrimental effects that will result in severe neurological and cognitive dysfunction in MS patients.

The still hypothetical model for MS pathogenesis described here is likely describing part of the *inside-out* process and, most importantly, it does not reject the argument for the presence of immune-mediated neuroinflammation in MS. Instead, it aims to elucidate a novel mechanism that may be implicated in driving the onset of the disease. If the proposed model were to reflect the driving mechanisms of progressive degeneration and perhaps, the causative initiator of MS, antioxidant therapies could provide novel therapeutic interventions for MS patients (Adamczyk and Adamczyk-Sowa, 2016). Over-time assessment of metabolite levels of both aerobic and glycolytic energy production in patients with RRMS and PPMS may help to resolve the role that mitochondrial dysfunction and OS play in MS. Upcoming cell-based approaches for MS should also take into consideration the crucial role that a dysfunctional and highly stressed environment may play in the mechanisms of tissue repair (Witherick et al., 2010). Hence, the direct targeting of OS processes and mitochondrial dysfunction may provide a more suitable microenvironment for successful stem cell transplantations or remyelination therapies.

AUTHOR CONTRIBUTIONS

AL and GS conceived the study. TB performed the literature research. GS and AL provided critical revision to the manuscript. TB wrote the manuscript with input from all other authors. All authors contributed to the article and approved the submitted version.

REFERENCES

Aboul-Enein, F., Rauschka, H., Kornek, B., Stadelmann, C., Stefferl, A., Brück, W., et al. (2003). Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. *J. Neuropathol. Exp. Neurol.* 62, 25–33. doi: 10.1093/jnen/62.1.25

Adamczyk, B., and Adamczyk-Sowa, M. (2016). New insights into the role of oxidative stress mechanisms in the pathophysiology and treatment of multiple sclerosis. *Oxid. Med. Cell Longev.* 2016:1973834. doi: 10.1155/2016/1973834

Baranzini, S. E., Mudge, J., Van Velkinburgh, J. C., Khankhanian, P., Khrebtukova, I., Miller, N. A., et al. (2010). Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature* 464, 1351–1356. doi: 10.1038/nature08990

Barnett, M. H., and Prineas, J. W. (2004). Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol.* 55, 458–468. doi: 10.1002/ana.20016

Barnett, M. H., Parratt, J. D. E., Pollard, J. D., and Prineas, J. W. (2009). MS: is it one disease? *Int. MS J.* 16, 57–65.

Basria, S. M. N., Mydin, R., and Okekpa, S. I. (2019). "Reactive oxygen species, cellular redox homeostasis and cancer", in *Homeostasis – An Integrated Vision*, eds Fernanda Lasakosvitsch Castanho and Sergio Dos Anjos Garnes (London, UK: IntechOpen), 123–141. doi: 10.5772/intechopen.76096

Betteridge, D. J. (2000). What is oxidative stress. *Metabolism* 2, 3–8. doi: 10.1016/S0026-0495(00)80077-3

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., and Crowe, S. E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol. Rev.* 94, 329–354. doi: 10.1152/physrev.00040.2012

Bjartmar, C., Wujek, J. R., and Trapp, B. D. (2003). Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *J. Neurol. Sci.* 206, 165–171. doi: 10.1016/s0022-510x(02)00069-2

Brinar, V. V., and Braun, B. (2013). Challenges in multiple sclerosis; how to define occurence of progression. *Clin. Neurol. Neurosurg.* 115, S30–S34. doi: 10.1016/j.clineuro.2013.09.017

Brück, W., and Stadelmann, C. (2003). Inflammation and degeneration in multiple sclerosis. *Neurol. Sci.* 24, S265–S267. doi: 10.1007/s10072-003-0170-7

Calabrese, M., Magliozzi, R., Ciccarelli, O., Geurts, J. J. G., Reynolds, R., and Martin, R. (2015). Exploring the origins of grey matter damage in multiple sclerosis. *Nat. Rev. Neurosci.* 16, 147–158. doi: 10.1038/nrn3900

Campbell, G. R., Worrall, J. T., and Mahad, D. J. (2014). The central role of mitochondria in axonal degeneration in multiple sclerosis. *Mult. Scler.* 20, 1806–1813. doi: 10.1177/1352458514544537

Caprariello, A. V., Rogers, J. A., Morgan, M. L., Hoghooghi, V., Plemel, J. R., Koebel, A., et al. (2018). Biochemically altered myelin triggers autoimmune demyelination. *Proc. Natl. Acad. Sci. U S A* 115, 5528–5533. doi: 10.1073/pnas.1721115115

Carvalho, A. N., Lim, J. L., Nijland, P. G., Witte, M. E., and van Horssen, J. (2014). Glutathione in multiple sclerosis: more than just an antioxidant? *Mult. Scler.* 20, 1425–1431. doi: 10.1177/1352458514533400

Chong, S. Y., Rosenberg, S. S., Fancy, S. P., Zhao, C., Shen, Y.-A. A., Hahn, A. T., et al. (2012). Neurite outgrowth inhibitor nogo-a establishes spatial segregation and extent of oligodendrocyte myelination. *Proc. Natl. Acad. Sci. U S A* 109, 1299–1304. doi: 10.1073/pnas.1113540109

Compston, A., and Coles, A. (2002). Multiple sclerosis. *Lancet* 359, 1221–1231. doi: 10.1016/S0140-6736(02)08220-X

Czaja, M. J. (2001). TNF toxicity--death from caspase or cathepsin, that is the question. *Hepatology* 34, 844–846. doi: 10.1053/jhep.2001.0340844

DeLuca, G. C., Ebers, G. C., and Esiri, M. M. (2004). Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts. *Brain* 127, 1009–1018. doi: 10.1093/brain/awh118

Dendrou, A. A., Fugger, L., and Friese, M. A. (2015). Immunopathology of multiple sclerosis. *Nat. Rev. Immunol.* 15, 545–558. doi: 10.1038/nri3871

Edgar, J. M., McCulloch, M. C., Montague, P., Brown, A. M., Thilemann, S., Pratola, L., et al. (2010). Demyelination and axonal preservation in a transgenic mouse model of pelizaeus-merzbacher disease. *EMBO Mol. Med.* 2, 42–50. doi: 10.1002/emmm.200900057

Fünfschilling, U., Supplie, L. M., Mahad, D., Boretius, S., Saab, A. S., Edgar, J., et al. (2012). Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* 485, 517–521. doi: 10.1038/nature11007

Fischer, M. T., Sharma, R., Lim, J. L., Haider, L., Frischer, J. M., Drexhage, J., et al. (2012). NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. *Brain* 135, 886–899. doi: 10.1093/brain/aws012

Franklin, R. J. M., and ffrench-Constant, C. (2008). Remyelination in the CNS: from biology to therapy. *Nat. Rev. Neurosci.* 9, 839–855. doi: 10.1038/nrn2480

Franklin, R. J. M., ffrench-Constant, C., Edgar, J. M., and Smith, K. J. (2012). Neuroprotection and repair in multiple sclerosis. *Nat. Rev. Neurol.* 8, 624–634. doi: 10.1038/nrneurol.2012.200

Friese, M. A., Schattling, B., and Fugger, L. (2014). Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat. Rev. Neurol.* 10, 225–238. doi: 10.1038/nrneurol.2014.37

Gaitán, M. I., De Alwis, M. P., Sati, P., Nair, G., and Reich, D. S. (2013). Multiple sclerosis shrinks intralesional, and enlarges extralesional, brain parenchymal veins. *Neurology* 80, 145–151. doi: 10.1212/WNL.0b013e31827b916f

Geurts, J. J. G., and Barkhof, F. (2008). Grey matter pathology in multiple sclerosis. *Lancet Neurol.* 7, 841–851. doi: 10.1016/S1474-4422(08)70191-1

Goldberg, M. P., and Ransom, B. R. (2003). New light on white matter. *Stroke* 34, 330–332. doi: 10.1161/01.str.0000054048.22626.b9

Griffiths, I., Klugmann, M., Anderson, T., Yool, D., Thomson, C., Schwab, M. H., et al. (1998). Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 280, 1610–1613. doi: 10.1126/science.280.5369.1610

Guo, C., Sun, L., Chen, X., and Zhang, D. (2013). Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen. Res.* 8, 2003–2014. doi: 10.3969/j.issn.1673-5374.2013.21.009

Guttmann, C. R. G., Rousset, M., Roch, J. A., Hannoun, S., Durand-Dubief, F., Belaroussi, B., et al. (2016). Multiple sclerosis lesion formation and early evolution revisited: a weekly high-resolution magnetic resonance imaging study. *Mult. Scler.* 22, 761–769. doi: 10.1177/1352458515600247

Haider, L. (2015). Inflammation, iron, energy failure, and oxidative stress in the pathogenesis of multiple sclerosis. *Oxid. Med. Cell Longev.* 2015:725370. doi: 10.1155/2015/725370

Hawker, K. (2011). Progressive multiple sclerosis: characteristics and management. *Neurol. Clin.* 29, 423–434. doi: 10.1016/j.ncl.2011.01.002

Henderson, A. P., Barnett, M. H., Parratt, J. D., and Prineas, J. W. (2009). Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann. Neurol.* 66, 739–753. doi: 10.1002/ana.21800

Klaver, R., De Vries, H. E., Schenk, G. J., and Geurts, J. J. G. (2013). Grey matter damage in multiple sclerosis: a pathology perspective. *Prion* 7, 66–75. doi: 10.4161/pri.23499

Kroemer, G., and Jäättelä, M. (2005). Lysosomes and autophagy in cell death control. *Nat. Rev. Cancer* 5, 886–897. doi: 10.1038/nrc1738

La Rocca, C., Carbone, F., De Rosa, V., Colamatteo, A., Galgani, M., Perna, F., et al. (2017). Immunometabolic profiling of t cells from patients with relapsing-remitting multiple sclerosis reveals an impairment in glycolysis and mitochondrial respiration. *Metabolism* 77, 39–46. doi: 10.1016/j.metabol.2017.08.011

Lappe-Siefke, C., Goebbels, S., Gravel, M., Nicksch, E., Lee, J., Braun, P. E., et al. (2003). Disruption of cnp1 uncouples oligodendroglial functions in axonal support and myelination. *Nat. Genet.* 33, 366–374. doi: 10.1038/ng1095

Lassmann, H. (2013). Pathology and disease mechanisms in different stages of multiple sclerosis. *J. Neurol. Sci.* 333, 1–4. doi: 10.1016/j.jns.2013.05.010

Lassmann, H., and van Horssen, J. (2011). The molecular basis of neurodegeneration in multiple sclerosis. *FEBS Lett.* 585, 3715–3723. doi: 10.1016/j.febslet.2011.08.004

Lassmann, H., van Horssen, J., and Mahad, D. (2012). Progressive multiple sclerosis: pathology and pathogenesis. *Nat. Rev. Neurol.* 8, 647–656. doi: 10.1038/nrneurol.2012.168

Lee, Y., Morrison, B. M., Li, Y., Lengacher, S., Farah, M. H., Hoffman, P. N., et al. (2012). Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* 487, 443–448. doi: 10.1038/nature11314

Lin, M. T., and Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443, 787–795. doi: 10.1038/nature05292

Lopes Pinheiro, M. A., Kooij, G., Mizee, M. R., Kamermans, A., Enzmann, G., Lyck, R., et al. (2016). Immune cell trafficking across the barriers of the central nervous system in multiple sclerosis and stroke. *Biochim. Biophys. Acta* 1862, 461–471. doi: 10.1016/j.bbadis.2015.10.018

Lubetzki, C., and Stankoff, B. (2014). Demyelination in multiple sclerosis. *Handb. Clin. Neurol.* 122, 89–99. doi: 10.1016/B978-0-444-52001-2.00004-2

Lubos, E., Loscalzo, J., and Handy, D. E. (2011). Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid. Redox Signal.* 15, 1957–1997. doi: 10.1089/ars.2010.3586

Mahad, D. H., Trapp, B. D., and Lassmann, H. (2015). Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol.* 14, 183–193. doi: 10.1016/S1474-4422(14)70256-X

Mahad, D., Lassmann, H., and Turnbull, D. (2008). Review: mitochondria and disease progression in multiple sclerosis. *Neuropathol. Appl. Neurobiol.* 34, 577–589. doi: 10.1111/j.1365-2990.2008.00987.x

Mews, I., Bergmann, M., Bunkowski, S., Gullotta, F., and Brück, W. (1998). Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. *Mult. Scler.* 4, 55–62. doi: 10.1177/135245859800400203

Micu, I., Plemel, J. R., Caprariello, A. V., Nave, K.-A., and Stys, P. K. (2018). Axo-myelinic neurotransmission: a novel mode of cell signalling in the central nervous system. *Nat. Rev. Neurosci.* 19, 49–58. doi: 10.1038/nrn.2017.128

Micu, I., Plemel, J. R., Lachance, C., Proft, J., Hansen, A. J., Cummins, K., et al. (2016). The molecular physiology of the axo-myelinic synapse. *Exp. Neurol.* 276, 41–50. doi: 10.1016/j.expneurol.2015.10.006

Nave, K. A. (2010). Myelination and support of axonal integrity by glia. *Nature* 468, 244–252. doi: 10.1038/nature09614

Nikić, I., Merkler, D., Sorbara, C., Brinkoetter, M., Bareyre, F. M., Brück, W., et al. (2011). A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. *Nat. Med.* 17, 495–499. doi: 10.1038/nm.2324

Noseworthy, J. H., Lucchinetti, C., Rodriguez, M., and Weinshenker, B. G. (2000). Multiple sclerosis. *N. Engl J. Med.* 343, 938–952. doi: 10.1056/NEJM200009283431307

Ohl, K., Tenbrock, K., and Kipp, M. (2016). Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Exp. Neurol.* 277, 58–67. doi: 10.1016/j.expneurol.2015.11.010

Ortiz, G. G., Flores-Alvarado, L. J., Pacheco-Moisés, F. P., Mireles-Ramírez, M. A., González-Renovato, E. D., Sánchez-López, A. L., et al. (2016). Cross-talk between glial cells and neurons: relationship in multiple sclerosis. *Clin. Case Rep. Rev.* 2, 565–571. doi: 10.15761/CCRR.1000276

Pham-Huy, L. A., He, H., and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* 4, 89–96.

Poerwoatmodjo, A., Schenk, G. J., Geurts, J. J. G., and Luchicchi, A. (2020). Cysteine proteases and mitochondrial instability: a possible vicious cycle in MS myelin? *Front. Cell Neurosci.* 14:612383. doi: 10.3389/fncel.2020.612383

Rodriguez, M., and Scheithauer, B. (1994). Ultrastructure of multiple sclerosis. *Ultrastruct. Pathol.* 18, 3–13. doi: 10.3109/01913129409016267

Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C. A., Patsopoulos, N. A., Moutsianas, L., et al. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219. doi: 10.1038/nature10251

Schnaar, R. L., and Lopez, P. H. H. (2009). Myelin-associated glycoprotein and its axonal receptors. *J. Neurosci. Res.* 87, 3267–3276. doi: 10.1002/jnr.21992

Seewann, A., Vrenken, H., van der Valk, P., Blezer, E. L. A., Knol, D. L., Castelijns, J. A., et al. (2009). Diffusely abnormal white matter in chronic multiple sclerosis: imaging and histopathologic analysis. *Arch. Neurol.* 66, 601–609. doi: 10.1001/archneurol.2009.57

Simons, M., Misgeld, T., and Kerschensteiner, M. (2014). A unified cell biological perspective on axon-myelin injury. *J. Cell Biol.* 206, 335–345. doi: 10.1083/jcb.201404154

Singh, S., Metz, I., Amor, S., van der Valk, P., Stadelmann, C., and Brück, W. (2013). Microglial nodules in early multiple sclerosis white matter are associated with degenerating axons. *Acta Neuropathol.* 125, 595–608. doi: 10.1007/s00401-013-1082-0

Strijbis, E. M., Inkster, B., Vounou, M., Naegelin, Y., Kappos, L., Radue, E.-W., et al. (2013). Glutamate gene polymorphisms predict brain volumes in multiple sclerosis. *Mult. Scler.* 19, 281–288. doi: 10.1177/1352458512454345

Stys, P. K. (2011). The axo-myelinic synapse. *Trends Neurosci.* 34, 393–400. doi: 10.1016/j.tins.2011.06.004

Stys, P. K. (2013). Pathoetiology of multiple sclerosis: are we barking up the wrong tree? *F1000Prime Rep.* 5:20. doi: 10.12703/P5-20

Stys, P. K., and Tsutsui, S. (2019). Recent advances in understanding multiple sclerosis. *F1000Res* 8:F1000. doi: 10.12688/f1000research.20906.1

Stys, P. K., Zamponi, G. W., van Minnen, J., and Geurts, J. J. G. (2012). Will the real multiple sclerosis please stand up? *Nat. Rev. Neurosci.* 13, 507–514. doi: 10.1038/nrn3275

Su, K. G., Banker, G., Bourdette, D., and Forte, M. (2009). Axonal degeneration in multiple sclerosis: the mitochondrial hypothesis. *Curr. Neurol. Neurosci. Rep.* 9, 411–417. doi: 10.1007/s11910-009-0060-3

Traka, M., Podojil, J. R., McCarthy, D. P., Miller, S. D., and Popko, B. (2016). Oligodendrocyte death results in immune-mediated CNS demyelination. *Nat. Neurosci.* 19, 65–74. doi: 10.1038/nn.4193

Trapp, B. D., and Nave, K. A. (2008). Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 31, 247–269. doi: 10.1146/annurev.neuro.30.051606.094313

Trapp, B. D., Peterson, J., Ransohoff, R. M., Rudick, R., Mörk, S., and Bö, L. (1998). Axonal transection in the lesions of multiple sclerosis. *N. Engl J. Med.* 338, 278–285. doi: 10.1056/NEJM199801293380502

Trevisiol, A., Saab, A. S., Winkler, U., Marx, G., Imamura, H., Möbius, W., et al. (2017). Monitoring ATP dynamics in electrically active white matter tracts. *eLife* 6:e24241. doi: 10.7554/eLife.24241

Tsutsui, S., and Stys, P. K. (2013). Metabolic injury to axons and myelin. *Exp. Neurol.* 246, 26–34. doi: 10.1016/j.expneurol.2012.04.016

Witherick, J., Wilkins, A., Scolding, N., and Kemp, K. (2010). Mechanisms of oxidative damage in multiple sclerosis and a cell therapy approach to treatment. *Autoimmune Dis.* 2011:164608. doi: 10.4061/2011/164608

Witte, M. E., Geurts, J. J. G., de Vries, H. E., van der Valk, P., and van Horssen, J. (2010). Mitochondrial dysfunction: a potential link between neuroinflammation and neurodegeneration? *Mitochondrion* 10, 411–418. doi: 10.1016/j.mito.2010.05.014

Witte, M. E., Mahad, D. J., Lassmann, H., and van Horssen, J. (2014). Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. *Trends Mol. Med.* 20, 179–187. doi: 10.1016/j.molmed.2013.11.007

Witte, M. E., Schumacher, A.-M., Mahler, C. F., Bewersdorf, J. P., Lehmitz, J., Scheiter, A., et al. (2019). Calcium influx through plasma-membrane nanoruptures drives axon degeneration in a model of multiple sclerosis. *Neuron* 101, 615.e5–624.e5. doi: 10.1016/j.neuron.2018.12.023

Yin, X., Crawford, T. O., Griffin, J. W., Tu, P. H., Lee, V. M. Y., Li, C., et al. (1998). Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. *J. Neurosci.* 18, 1953–1962. doi: 10.1523/JNEUROSCI.18-06-01953

Zong, W.-X., and Thompson, C. B. (2006). Necrotic death as a cell fate. *Genes Dev.* 20, 1–15. doi: 10.1101/gad.1376506

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