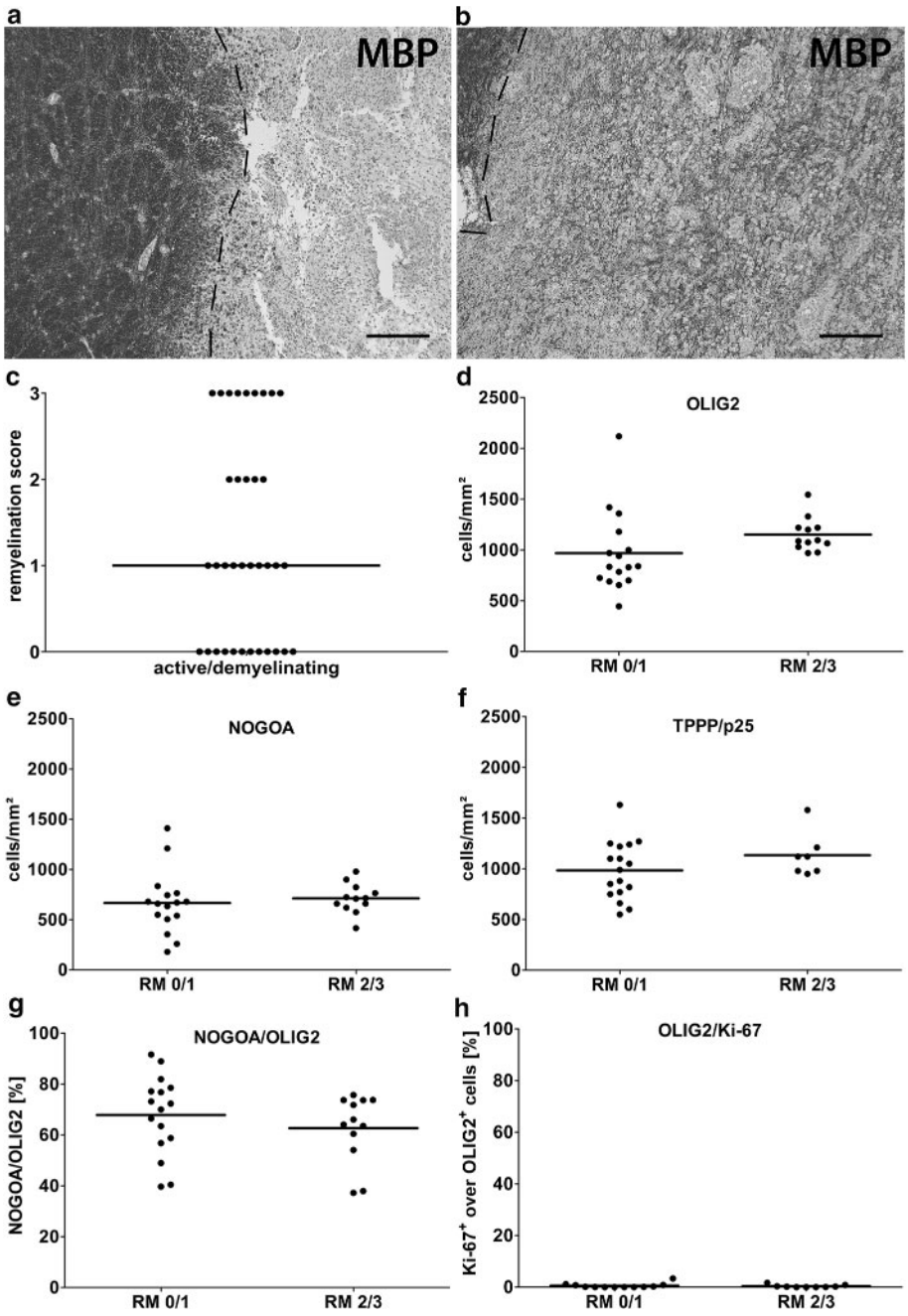


Fig. 3 No differences in oligodendroglial cell numbers in active/demyelinating lesions with and without marked remyelination. **a**, **b** A lesion with no remyelination (score 0) is shown in **a**, whereas in **b,** a lesion with marked remyelination (score 3) is displayed (IHC for MBP). **c** In 14 out of 36 (=39%) of the active/demyelinating lesions, we observed marked remyelination (score 2/3). **d–f** Quantification of $OLIG2^+$, $NOGOA^+$ and $TPPP/p25^+$ cells in active/demyelinating lesions with (score 2/3) and without (score 0/1) marked remyelination. **g** No significant difference in the ratio of $NOGOA^+$ cells over $OLIG2^+$ in lesions with and without marked remyelination. **h** Double IHC for Ki-67 and OLIG2 demonstrated that only few $OLIG2^+$ cells express Ki-67**.** Scale bars in **a** and **b**: 200 µm. *MBP* myelin basic protein, *OLIG2* oligodendrocyte transcription factor 2, *TPPP/p25* tubulin polymerization promoting protein, *active/demyelinating* active/demyelinating lesions, *RM 0/1* remyelination score 0 or 1, *RM 2/3* remyelination score 2 or 3

of less than 50% of the lesion area (score 1). The difference in the extent of remyelination between mixed and active as well as inactive lesions was highly significant ($p < 0.001$) (Fig. 4c).

Higher percentages of $TMEM119^+$ and $iNOS^+$ myeloid cells are associated with less remyelination in mixed lesions

To investigate whether the inflammatory milieu may influence the outcome of remyelination, we examined the composition of inflammatory infiltrates in active, mixed, and inactive lesions (Fig. 5a–f). Myeloid cells were the dominating inflammatory cell population in all lesion types; however, the numbers of myeloid cells in inactive lesions as well as in the center of mixed lesions were significantly lower compared to active and the rim of mixed lesions (Fig. 5d). Highest numbers of T and B cells were found in active lesions (Fig. 5e, f).

Due to the dominance of the myeloid cell population and based on a plethora of literature describing either detrimental or beneficial effects of myeloid cells on remyelination, we hypothesized that those myeloid cells may determine the outcome of remyelination. We focused our subsequent analyses on

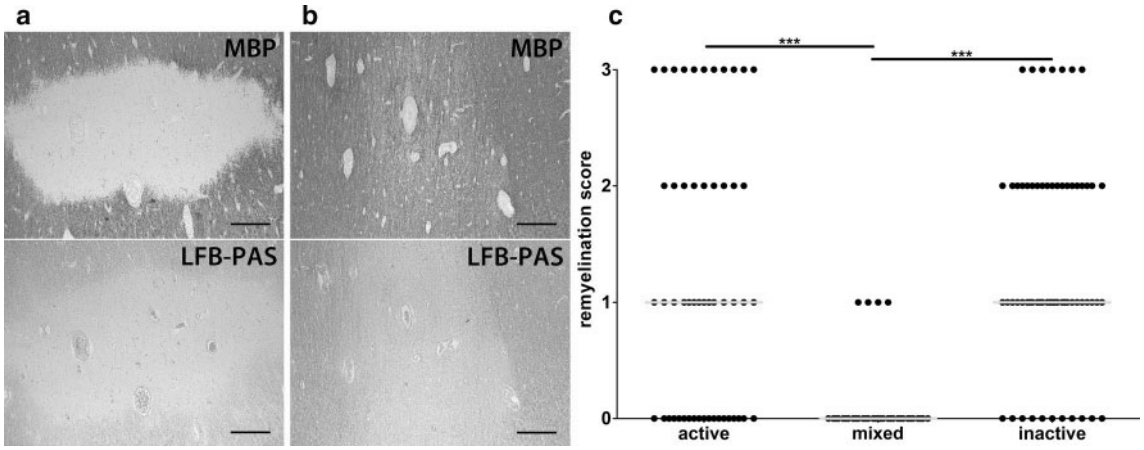


Fig. 4 Almost complete lack of remyelination in mixed lesions. **a**, **b** Pictures display an inactive lesion with no remyelination (score 0) (**a**), whereas **b** shows a completely remyelinated shadow plaque (score 3). Upper panels in **a** and **b** show IHC for MBP, lower panels in **a** and **b** display LFB-PAS staining. **c** Semiquantitative analysis of remyelination reveals an almost complete lack of remyelination in mixed lesions (**c**). Scale bars in **a** and **b**: 500 µm. *MBP* myelin basic protein, *LFB-PAS* luxol fast blue–periodic acid Schiff; *active* active lesions, *mixed* mixed active/inactive lesions, *inactive* inactive lesions

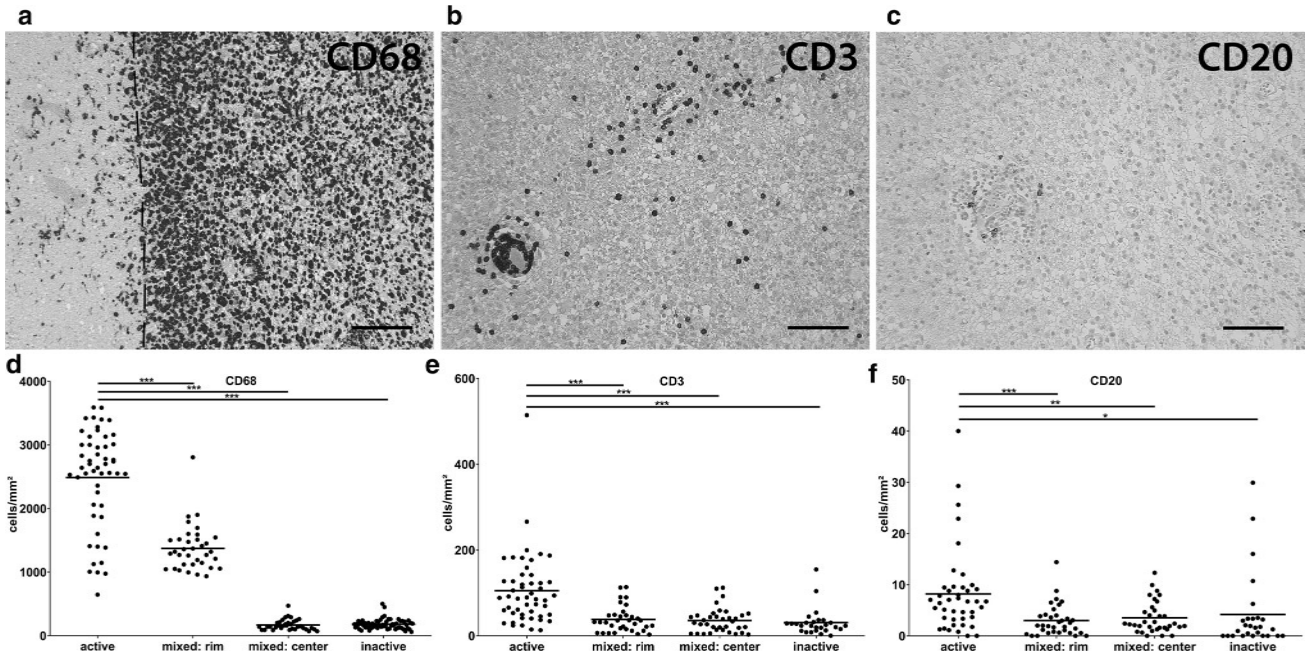


Fig. 5 Inflammatory environment in different lesion types. **a–c** IHC demonstrates numerous $CD68^+$ myeloid cells, fewer $CD3^+$ T cells and few $CD20^+$ B cells in an active lesion. All pictures are taken from the same active lesion. **d–f** Quantification of $CD68^+$, $CD3^+$ and $CD20^+$ cells in different lesion types. Please note the different y-axes. Scale bar in **a**: 200 µm, scale bars in **b** and **c**: 100 µm. *CD3/20/68* cluster of differentiation 3/20/68, *active* active lesions, *mixed: rim* rim of mixed active/inactive lesions, *mixed: center* center of mixed active/inactive lesions, *inactive* inactive lesions

active lesions and the rim of mixed lesions, since the center of mixed lesions as well as inactive lesions was almost completely depleted of myeloid cells. To characterize the myeloid cell population in more detail, we stained for TMEM119, a marker of homeostatic microglia, iNOS, a pro-inflammatory marker as well as CD163 and CD206, two markers associated with an anti-inflammatory phenotype of myeloid cells (Fig. 6a–d). The highest absolute number of myeloid cells was identified by CD163 (mean ± SEM: 906 ± 70) followed by TMEM119 (mean ± SEM: 640 ± 36) and iNOS (mean ± SEM: 468 ± 38), whereas fewer myeloid cells expressed CD206 (mean + SEM: 128 ± 12) (Supplementary Fig. 2a–e, online resource). When

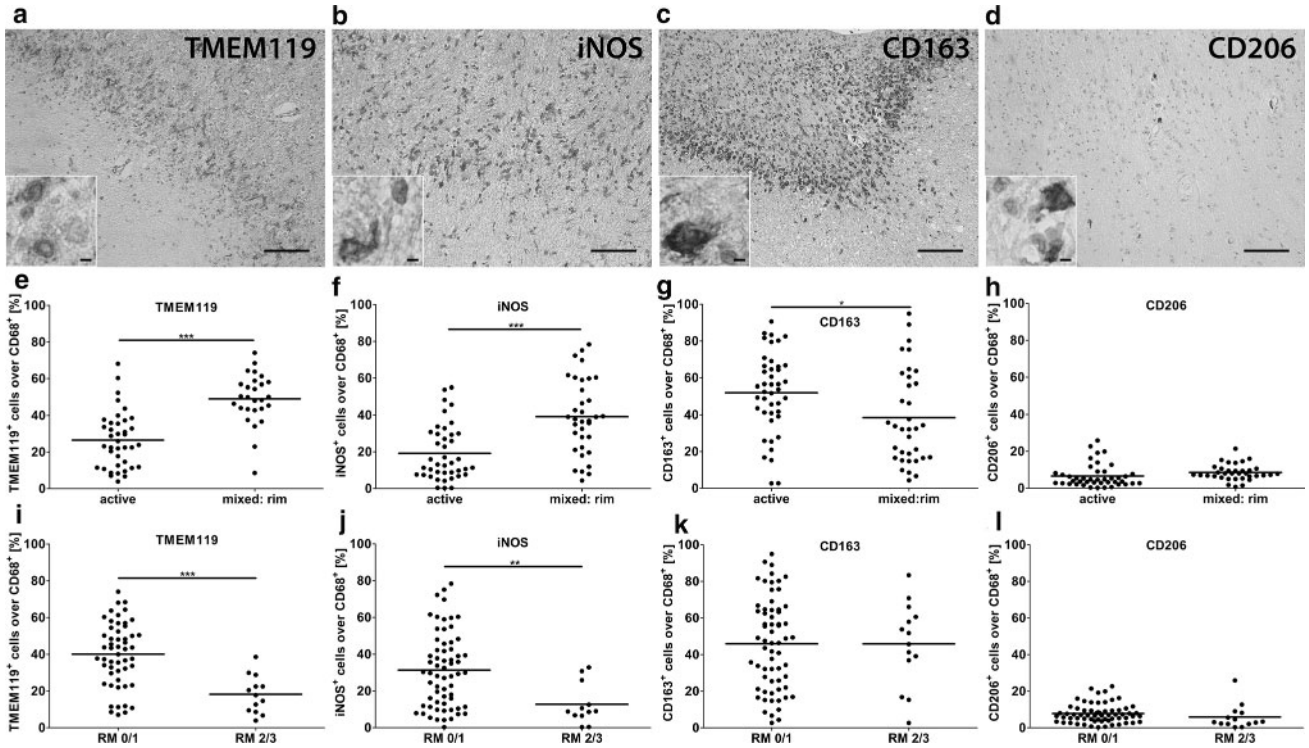


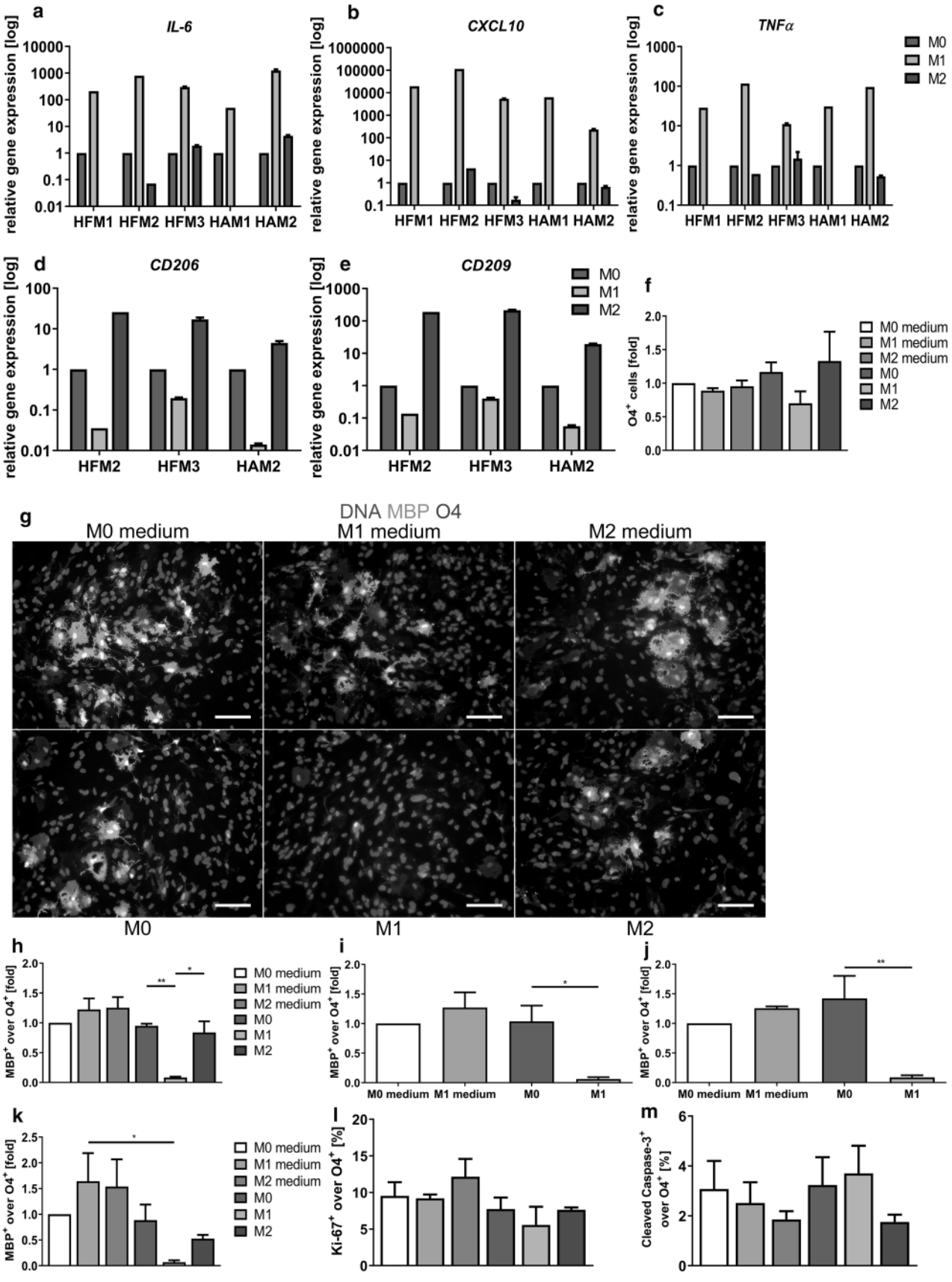
Fig. 6 Relative increase in the numbers of TMEM119$^+$ and iNOS$^+$ myeloid cells in the rim of mixed lesions. **a–d** IHC for TMEM119, iNOS, CD163 and CD206 was performed. Inserts show labelled cells in higher magnification. **e–g** Quantification demonstrated a significant relative increase in the ratio of TMEM119$^+$/CD68$^+$ and iNOS$^+$/CD68$^+$ myeloid cells and a relative decrease of CD163$^+$/CD68$^+$ myeloid cells in the rim of mixed lesions compared to active lesions. **h** No differences in the number of CD206$^+$ over CD68$^+$ cells were observed between active lesions and the rim of mixed lesions. **i**, **j** Comparably, we observed a relative increase in the ratio of TMEM119$^+$/CD68$^+$ and iNOS$^+$/CD68$^+$ in lesions with limited (score 0/1) compared to lesions with marked remyelination (score 2/3). **k**, **l** No differences were found between lesions with and without marked remyelination with respect to the ratio of CD163$^+$/CD68$^+$ and CD203$^+$/CD68$^+$ cells. Scale bars in **a**, **b** and **d**: 100 µm, scale bar in **c**: 200 µm, scale bar in insert in **a–d**: 6.25 µm. *TMEM119* transmembrane protein 119, *iNOS* inducible nitric oxide synthase, *CD68/163/206* cluster of differentiation 68/163/206, *active* active lesions, *mixed: rim* rim of mixed active/inactive lesions, *RM 0/1* remyelination score 0 or 1, *RM 2/3* remyelination score 2 or 3

analyzing the ratio of TMEM119$^+$/CD68$^+$, iNOS$^+$/CD68$^+$ and CD163$^+$/CD68$^+$ cells, we saw a significant increase in the percentage of TMEM119$^+$ and iNOS$^+$ over CD68$^+$ myeloid cells in the rim of mixed lesions compared to active lesions (Fig. 6e, f). The percentage of CD163$^+$ cells was significantly reduced in the rim of mixed lesions, whereas no difference in the number of CD206$^+$ myeloid cells was observed (Fig. 6g, h). Furthermore, when comparing the ratio of TMEM119$^+$, iNOS$^+$, CD163$^+$ and CD206$^+$ over CD68$^+$ cells in active and mixed lesions with limited (score 0/1) or marked remyelination (score 2/3), we observed a relative increase in TMEM119$^+$ and iNOS$^+$ myeloid cells in lesions with limited remyelination.

In summary, these data demonstrate a relative increase of TMEM119$^+$ homeostatic microglia and iNOS$^+$ pro-inflammatory myeloid cells in the rim of mixed lesions. Furthermore, a relative increase in TMEM119$^+$ and iNOS$^+$ myeloid cells is associated with reduced remyelination in active and mixed lesions.

Supernatants of M1, but not M0 or M2 polarized microglia inhibit terminal differentiation of hiOL

To further elucidate the impact of pro-inflammatory myeloid cells on oligodendroglial differentiation, we investigated the role of supernatants of activated microglia on hiOL [22]. Fetal and adult human primary microglia were stimulated for 48 h with either LPS and IFNγ to promote an M1 phenotype or with IL-4 and IL-13 to induce an M2 phenotype. In total, we obtained microglia supernatants from three fetal (HFM1-3) and two adult (HAM1, 2) donors. For validation of successful polarization, we determined the expression of typical M1 (*IL-6, CXCL10, TNFα*) and M2 cytokines (*CD206* and *CD209*) by qRT-PCR (Fig. 7a–e). Supernatants as well as the appropriate medium without the supernatants (controls) were added to differentiating hiOL from three different donors in a dilution of 1:10 from day 4 to 21 of differentiation to examine the effect on early differentiation. No differences in the percentage of O4$^+$ cells were observed when comparing appropriate medium controls and


a
IL-6
relative gene expression [log]
HFM1 HFM2 HFM3 HAM1 HAM2
b
CXCL10
c
TNFα
M0
M1
M2
d
CD206
HFM2 HFM3 HAM2
e
CD209
f
O4+ cells [fold]
M0 medium
M1 medium
M2 medium
g
DNA MBP O4
M0 medium
M1 medium
M2 medium
M0
M1
M2
h
MBP+ over O4+ [fold]
i
j
M0 medium M1 medium M0 M1
k
l
Ki-67+ over O4+ [%]
m
Cleaved Caspase-3+ over O4+ [%]

◂**Fig. 7** Supernatants of primary human M1, but not M2 or M0 polarized microglia inhibit the terminal differentiation of hiOL. Every experiment analyzing the effect of microglia supernatants on hiOL was performed with hiOL derived from three different donors. **a–e** To confirm successful polarization into M1 and M2 microglia, qRT-PCR was performed for *IL-6, CXCL10* and *TNFα* (M1) and *CD206* and *CD209* (M2). **f** Differentiation of hiOL in the presence of supernatants from M0, M1 or M2 polarized microglia from one fetal donor (HFM2) from day 4 to 21 resulted in no significant differences in the percentage of $O4^+$ hiOL when comparing the effect of different supernatants with appropriate controls. **g**, **h** Culturing O4 sorted hiOL in the presence of supernatants from M1, but not M0 or M2 polarized microglia from one fetal donor (HFM3) from day 21 to 35 impaired significantly the differentiation of $O4^+$ hiOL into MBP^+ mature oligodendrocytes. **i–k** This was confirmed using supernatants from M1 and M0 polarized microglia from another fetal (HFM1) and one adult donor (HAM1) as well as supernatants from M0, M1 and M2 polarized microglia from a second adult donor (HAM2). **l** Analysis of percentages of actively dividing hiOL ($Ki\text{-}67^+$ over $O4^+$ cells) in the presence of supernatants from M0, M1 or M2 polarized microglia from one fetal donor (HFM2) from day 21 to 35 of differentiation did not reveal any significant differences. **m** Percentages of apoptotic hiOL (cleaved caspase 3^+ over $O4^+$ cells) in the presence of supernatants from M0, M1 or M2 polarized microglia from one fetal donor (HFM3) from day 21 to 35 of differentiation were not significantly altered. Scale bar in **g**: 100 µm. *hiOL* human iPSC-derived oligodendrocytes, *HFM* human fetal microglia, *HAM* human adult microglia, *IL-6* interleukin 6, *CXCL10* C-X-C motif chemokine 10, *TNFα* tumor necrosis factor alpha, *CD206/CD209* cluster of differentiation 206/209, *MBP* myelin basic protein

supernatants (Fig. 7f) as assessed by flow cytometry for the oligodendroglial marker O4 which is expressed by immature and mature oligodendrocytes. To determine the effect of the supernatants on terminal differentiation, untreated $O4^+$ hiOL sorted at day 21 of differentiation were exposed to M0, M1, and M2 microglia supernatants or medium controls for 14 days (dilution 1:10). ICC for MBP revealed significantly reduced numbers of mature MBP^+ hiOL after application of M1 microglia supernatants but not M0 or M2 microglia supernatants from a fetal donor, while $O4^+$ hiOL could still be detected (Fig. 7g, h). These results were validated using another fetal as well as two adult donors (Fig. 7i–k). To elucidate whether the reduced presence of MBP^+ cells is caused by a decreased differentiation into mature hiOL or due to changes in cell death or proliferation, we analyzed the numbers of apoptotic and actively dividing hiOL by double ICC for cleaved caspase 3, respectively, Ki-67 and O4. Neither the percentage of apoptotic nor the percentage of actively dividing hiOL was significantly altered after application of microglia supernatants (Fig. 7l, m). Combined, these data demonstrate that supernatants from M1 polarized human microglia significantly inhibit the terminal differentiation of hiOL into mature MBP^+ oligodendrocytes but do not affect apoptosis or proliferation of hiOL.

Discussion

Promotion of remyelination represents a new and attractive treatment target in MS. In demyelinating animal models, the proliferation and differentiation of OPC are prerequisites for successful remyelination. In progressive disease stages, MS lesions display a relative preservation of OPC, but lack mature oligodendrocytes suggesting that a differentiation block contributes to remyelination failure in MS. This notion has been recently challenged by suggesting that not OPC, but mature oligodendrocytes are the major remyelinating cells in MS [34, 54, 71]. To further elucidate the mechanisms contributing to impaired remyelination in MS, we performed a detailed analysis of oligodendrocytes and remyelination in different MS lesion types. Our data suggest that different mechanisms contribute to impaired remyelination depending on lesion stage. In active/demyelinating lesions, remyelination occurs only in a subset of lesions despite the presence of high numbers of mature oligodendrocytes in all lesions indicating that an impaired formation of new myelin sheaths rather than a lack of mature oligodendrocytes contributes to insufficient remyelination in this lesion type. Furthermore, in mixed lesions, we observed an almost complete lack of remyelination and a relative increase of $TMEM119^+$ microglia cells and $iNOS^+$ pro-inflammatory myeloid cells. Supernatants of M1, but not M2 or M0 polarized microglia prevented terminal differentiation of hiOL indicating that pro-inflammatory microglia may contribute to impaired remyelination in mixed lesions.

The generally accepted view that OPC are prerequisite for successful remyelination is, among others, based on studies demonstrating that rodents in which proliferating oligodendrocytes were ablated by X-irradiation did not remyelinate [36]. Additionally, mature human oligodendrocytes transplanted into demyelinated and irradiated spinal cord lesions of rats did not contribute to remyelination [64]. One could speculate that X-irradiation may result in significant damage to the exposed tissue and other factors than the supposed incapability of mature oligodendrocytes may contribute to remyelination failure in these studies. However, in an elegant and more recent study, Crawford and colleagues found no evidence that pre-existing mature oligodendrocytes contribute to remyelination after toxin-induced demyelination using inducible reporter mouse lines and fate mapping [14]. Furthermore, a broad range of studies demonstrated that increased differentiation of OPC into mature myelinating oligodendrocytes results in accelerated remyelination in experimental animal models (for review see [25, 62]). Earlier histological studies using MS tissue samples demonstrated the presence of OPC and an almost complete absence of mature oligodendrocytes in mixed and inactive lesions [11, 39, 68, 70]. These findings together with the results from

animal studies were interpreted as evidence for an impaired oligodendroglial differentiation as contributing factor for remyelination failure in MS. This view has been challenged by experimental studies reporting mature oligodendrocytes being connected to mature and remyelinated myelin sheaths in a cat as well as in a non-human primate model of de- and remyelination [19]. Additionally, the results of a recent study measuring the integration of ^{14}C derived from nuclear testing into DNA of oligodendroglial lineage cells suggest that pre-existing oligodendrocytes and not proliferating OPC contribute to remyelination in MS [71]. Our data demonstrate the presence of remyelination and preservation of oligodendrocytes in highly inflammatory active/demyelinating lesions suggesting that oligodendrocytes are not the primary target of the immune attack in MS lesions. Furthermore, despite the presence of high numbers of mature oligodendrocytes, only a subset of lesions showed signs of remyelination. This indicates that not a differentiation block, but rather an impaired formation of new myelin sheaths contributes to remyelination failure in these lesions. Additionally, it raises the question which cells contributed to successful remyelination in active/demyelinating lesions with marked remyelination. We observed only a very low percentage of proliferating OPC in these lesions suggesting that these cells may not be major contributors to remyelination. Considering further the fact that OPC account for approximately 5% of the total oligodendroglial population and the relatively long time period required for differentiation of human OPC into mature oligodendrocytes [7, 12, 39], it appears unlikely that only recently differentiated OPC contributed to successful remyelination in active/demyelinating lesions, but instead favors a scenario in which also mature oligodendrocytes surviving the initial inflammatory attack contribute to remyelination. However, one has to keep in mind that Ki-67 only identifies cells which are actively dividing. The discrepancy between this scenario and experimental rodent studies might be explained by the fact that EAE, lysolecithine or cuprizone induced demyelination are characterized by a marked loss of mature oligodendrocytes [40, 49, 50] which is in contrast to our observations in active/demyelinating MS lesions. However, histology provides only a snapshot, and as long as no reliable markers for recently differentiated oligodendrocytes and remyelination are available, we will not be able to definitively clarify which cell population contributes to remyelination in MS. Importantly, our data also do not exclude the possibility that in other lesion types than active/demyelinating lesions, an oligodendroglial differentiation block contributes to impaired remyelination. Furthermore, remyelination is a complex process. It not only depends on the presence and absence of oligodendrocytes or its progenitors, but also requires axons susceptible for remyelination. Several studies for example have demonstrated that neuronal activity stimulates remyelination [3, 28, 55, 66]. Additionally, also astrocytes may influence the outcome of remyelination. They can be beneficial for remyelination by promotion of oligodendroglial differentiation and providing of cholesterol for new myelin sheaths, but have been also shown to impair remyelination by either activating remyelination inhibiting pathways such as the JAG1-NOTCH1 pathway or by modulating the extracellular matrix [10, 32, 35, 61, 72]. One also has to keep in mind that the biopsies were taken from patients with an untypical clinical presentation and, therefore, might not be representative for MS patients with a more typical disease onset. However, most of the patients diagnosed with an inflammatory demyelinating lesion consistent with MS, developed clinical definitive MS with a relapsing–remitting disease course [59].

The histological appearance of MS lesions changes over time. In acute and RRMS active lesions predominate; however, when MS progresses, the percentage of active lesions decreases, whereas the percentages of mixed and inactive lesions increase [26, 46]. Patients with a more severe disease course have a higher percentage of mixed lesions suggesting that mixed lesions contribute significantly to disease progression [46]. Interestingly, we observed an almost complete absence of remyelination in mixed lesions in contrast to active or inactive lesions. This is in line with an earlier publication by Luchetti and colleagues who reported an inverse correlation between mixed versus remyelinated lesions [46]. Remyelination is frequently occurring at the lesion border; however, mixed lesions are characterized by a rim of myeloid cells at the lesion border suggesting that tissue environment in the rim of mixed lesions prevents successful remyelination. Interestingly, we observed a relative increase in $TMEM119^+$ and $iNOS^+$ and a decrease of $CD163^+$ myeloid cells in the rim of mixed lesions compared to active lesions; however, one has also to keep in mind that TMEM119 is a homeostatic microglia marker whose expression decreases with activation [4]. In EAE, myeloid cells change from an $iNOS^+$ pro-inflammatory and tissue-destructive phenotype to an $iNOS^-$ tissue repair phenotype and this may be mediated by other immune cells, such as T and B cells [23, 43, 58]. This is in contrast to mixed MS lesions where at least a subset of myeloid cells preserves an $iNOS^+$ phenotype. To further understand consequences of pro-inflammatory myeloid cells for oligodendrocytes, we exposed hiOL to the supernatants of pro- and anti-inflammatory microglia. We observed a marked decrease in the terminal differentiation into MBP^+ oligodendrocytes after exposure to supernatants from pro-inflammatory human microglia, whereas supernatants from microglia polarized into an M0 or an M2 phenotype did not affect oligodendroglial differentiation. This is contrary to results from rodent studies in which supernatants from M0 and M1 polarized microglia had no effect on oligodendroglial differentiation, whereas the supernatants from M2 polarized microglia promoted oligodendroglial

differentiation [52]. Microvesicles isolated from either pro- or anti-inflammatory rat microglia both promoted the differentiation of rat oligodendrocytes [44]. These different results suggest that there might be significant differences between rodent and human oligodendrocytes with respect to the reaction to an inflammatory milieu. However, also the protocols for the polarization into pro- and anti-inflammatory microglial cells were slightly different and this might have also affected the outcome. Furthermore, other immune cells, such as T and B cells, have been reported to influence remyelination, either via direct effects on oligodendrocytes or by modulating myeloid cells [17].

We observed a comparable percentage of lesions with marked remyelination in active and inactive lesions. Therefore, it might be tempting to speculate that active lesions with marked remyelination become remyelinated inactive lesions, whereas active lesions lacking marked remyelination turn into mixed lesions and potentially afterwards into inactive lesions lacking remyelination. However, as mentioned above, histological studies provide only a snapshot and imaging studies demonstrated repeated waves of de- and remyelination in MS lesions [6]. Imaging markers for the different lesions types and longitudinal imaging studies are required to understand how lesions develop over time and how they contribute to disease progression.

In summary, our data suggest that the underlying causes for remyelination failure in MS are lesion stage dependent. Whereas in active/demyelinating lesions, lack of myelin sheath formation contributes to remyelination failure, in inactive and mixed lesions oligodendroglial loss and a hostile tissue environment may prevent successful remyelination. Since pharmacological approaches that focus exclusively on promotion of oligodendroglial differentiation to enhance remyelination might fail, treatment strategies targeting multiple of the steps required for successful remyelination, namely oligodendroglial proliferation, migration, differentiation, myelin sheath formation, and survival should be considered. Our data also suggest that promotion of remyelination in progressive MS in which mixed and inactive lesions dominate may require different treatment approaches compared to RRMS characterized by more active lesions. To understand the molecular mechanisms driving and preventing remyelination and to develop new treatment approaches which successfully promote remyelination in MS, better animal models are required which mimic the pathological hallmarks of the different lesion types.

Acknowledgements Open Access funding provided by Projekt DEAL. We thank Elke Hoffmann and Claudia Kemming (University Hospital Münster, Münster DE) for excellent technical assistance. This work was supported by the German Research Foundation (DFG CRC-TR-128B07 and CRC-TR-128Z02 to T.K.), Progressive MS Alliance (collaborative research network PA-1604-08492 (BRAVEinMS) to T.K., G.M. and J.A.), the National MS Society (RG-1801-30020 to T.K.), the Hertie Foundation (fellowship to K.H.) and the MedK, Medical Faculty Münster (fellowship to K.H.).

Author contributions TK initiated the project. KH, LS, and TK conceived and designed the study. KH and TK analyzed MS tissue samples. LS developed and performed the cell culture experiments and analyzed the data. NWK, MCJV, LH, and H generated and characterized the supernatants from inflammatory cells. CT analysed data. JA, GM, and IH discussed results and provided cell lines. KH, LS, and TK wrote the manuscript with critical insight and commentary from all co-authors.

Compliance with ethical standards

Conflict of interest T.K. has a pending patent application for the generation of human oligodendrocytes.

References

1. Adelman G, Rane SG, Villa KF (2013) The cost burden of multiple sclerosis in the United States: a systematic review of the literature. J Med Econ 16:639–647. https://doi.org/10.3111/13696998.2013.778268
2. Albert M, Antel J, Bruck W, Stadelmann C (2007) Extensive cortical remyelination in patients with chronic multiple sclerosis. Brain Pathol 17:129–138
3. Barres BA, Raff MC (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature 361:258–260. https://doi.org/10.1038/361258a0
4. Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB et al (2016) New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci USA 113:E1738–1746. https://doi.org/10.1073/pnas.1525528113
5. Bodini B, Veronese M, Garcia-Lorenzo D, Battaglini M, Poirion E, Chardain A et al (2016) Dynamic imaging of individual remyelination profiles in multiple sclerosis. Ann Neurol. https://doi.org/10.1002/ana.24620
6. Brown RA, Narayanan S, Arnold DL (2014) Imaging of repeated episodes of demyelination and remyelination in multiple sclerosis. Neuroimage Clin 6:20–25. https://doi.org/10.1016/j.nicl.2014.06.009
7. Butt A, Kiff J, Hubbard P, Berry M (2002) Synantocytes: new functions for novel NG2 expressing glia. J Neurocytol 31:551–565
8. Cadavid D, Balcer L, Galetta S, Aktas O, Ziemssen T, Vanopdenbosch L et al (2017) Safety and efficacy of opicinumab in acute optic neuritis (RENEW): a randomised, placebo-controlled, phase 2 trial. Lancet Neurol 16:189–199. https://doi.org/10.1016/S1474-4422(16)30377-5

9. Cadavid D, Mellion M, Hupperts R, Edwards KR, Calabresi PA, Drulovic J et al (2019) Safety and efficacy of opicinumab in patients with relapsing multiple sclerosis (SYNERGY): a randomised, placebo-controlled, phase 2 trial. Lancet Neurol 18:845–856. https://doi.org/10.1016/S1474-4422(19)30137-1
10. Cantuti-Castelvetri L, Fitzner D, Bosch-Queralt M, Weil MT, Su M, Sen P et al (2018) Defective cholesterol clearance limits remyelination in the aged central nervous system. Science. https://doi.org/10.1126/science.aan4183
11. Chang A, Tourtellotte WW, Rudick R, Trapp BD (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 346:165–173
12. Chanoumidou K, Mozafari S, Baron-Van Evercooren A, Kuhlmann T (2020) Stem cell derived oligodendrocytes to study myelin diseases. Glia 68:705–720. https://doi.org/10.1002/glia.23733
13. Chen JT, Collins DL, Atkins HL, Freedman MS, Arnold DL (2008) Magnetization transfer ratio evolution with demyelination and remyelination in multiple sclerosis lesions. Ann Neurol 63:254–262
14. Crawford AH, Tripathi RB, Foerster S, McKenzie I, Kougioumtzidou E, Grist M et al (2016) Pre-existing mature oligodendrocytes do not contribute to remyelination following toxin-induced spinal cord demyelination. Am J Pathol 186:511–516
15. Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15:545–558. https://doi.org/10.1038/nri3871
16. Deshmukh VA, Tardif V, Lyssiotis CA, Green CC, Kerman B, Kim HJ et al (2013) A regenerative approach to the treatment of multiple sclerosis. Nature 502:327–332
17. Dombrowski Y, O'Hagan T, Dittmer M, Penalva R, Mayoral SR, Bankhead P et al (2017) Regulatory T cells promote myelin regeneration in the central nervous system. Nat Neurosci 20:674–680. https://doi.org/10.1038/nn.4528
18. Duncan ID, Brower A, Kondo Y, Curlee JF Jr, Schultz RD (2009) Extensive remyelination of the CNS leads to functional recovery. Proc Natl Acad Sci USA 106:6832–6836
19. Duncan ID, Radcliff AB, Heidari M, Kidd G, August BK, Wierenga LA (2018) The adult oligodendrocyte can participate in remyelination. Proc Natl Acad Sci USA 115:E11807–E11816. https://doi.org/10.1073/pnas.1808064115
20. Durafourt BA, Moore CS, Blain M, Antel JP (2013) Isolating, culturing, and polarizing primary human adult and fetal microglia. Methods Mol Biol 1041:199–211. https://doi.org/10.1007/978-1-62703-520-0_19
21. Durafourt BA, Moore CS, Zammit DA, Johnson TA, Zaguia F, Guiot MC et al (2012) Comparison of polarization properties of human adult microglia and blood-derived macrophages. Glia 60:717–727. https://doi.org/10.1002/glia.22298
22. Ehrlich M, Mozafari S, Glatza M, Starost L, Velychko S, Hallmann AL et al (2017) Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. Proc Natl Acad Sci USA 114:E2243–E2252. https://doi.org/10.1073/pnas.1614412114
23. El Behi M, Sanson C, Bachelin C, Guillot-Noel L, Fransson J, Stankoff B et al (2017) Adaptive human immunity drives remyelination in a mouse model of demyelination. Brain 140:967–980. https://doi.org/10.1093/brain/awx008
24. Evans FL, Dittmer M, de la Fuente AG, Fitzgerald DC (2019) Protective and regenerative roles of T cells in central nervous system disorders. Front Immunol 10:2171. https://doi.org/10.3389/fimmu.2019.02171
25. Franklin RJM, Ffrench-Constant C (2017) Regenerating CNS myelin—from mechanisms to experimental medicines. Nat Rev Neurosci 18:753–769. https://doi.org/10.1038/nrn.2017.136
26. Frischer JM, Weigand SD, Guo Y, Kale N, Parisi JE, 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
27. Galloway DA, Gowing E, Setayeshgar S, Kothary R (2020) Inhibitory milieu at the multiple sclerosis lesion site and the challenges for remyelination. Glia 68:859–877. https://doi.org/10.1002/glia.23711
28. Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS et al (2014) Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. Science 344:1252304. https://doi.org/10.1126/science.1252304
29. Goldschmidt T, Antel J, Konig FB, Brück W, Kuhlmann T (2009) Remyelination capacity of the MS brain decreases with disease chronicity. Neurology 72:1914–1921
30. Green AJ, Gelfand JM, Cree BA, Bevan C, Boscardin WJ, Mei F et al (2017) Clemastine fumarate as a remyelinating therapy for multiple sclerosis (ReBUILD): a randomised, controlled, double-blind, crossover trial. Lancet 390:2481–2489. https://doi.org/10.1016/S0140-6736(17)32346-2
31. Hoftberger R, Fink S, Aboul-Enein F, Botond G, Olah J, Berki T et al (2010) Tubulin polymerization promoting protein (TPPP/p25) as a marker for oligodendroglial changes in multiple sclerosis. Glia 58:1847–1857. https://doi.org/10.1002/glia.21054
32. Houben E, Janssens K, Hermans D, Vandooren J, Van den Haute C, Schepers M et al (2020) Oncostatin M-induced astrocytic tissue inhibitor of metalloproteinases-1 drives remyelination. Proc Natl Acad Sci USA 117:5028–5038. https://doi.org/10.1073/pnas.1912910117
33. Irvine KA, Blakemore WF (2008) Remyelination protects axons from demyelination-associated axon degeneration. Brain 131:1464–1477
34. Jakel S, Agirre E, Mendanha Falcao A, van Bruggen D, Lee KW, Knuesel I et al (2019) Altered human oligodendrocyte heterogeneity in multiple sclerosis. Nature 566:543–547. https://doi.org/10.1038/s41586-019-0903-2
35. John GR, Shankar SL, Shafit-Zagardo B, Massimi A, Lee SC, Raine CS et al (2002) Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. Nat Med 8:1115–1121
36. Keirstead HS, Blakemore WF (1997) Identification of post-mitotic oligodendrocytes incapable of remyelination within the demyelinated adult spinal cord. J Neuropathol Exp Neurol 56:1191–1201
37. Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Brück W (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125:2202–2212
38. Kuhlmann T, Ludwin S, Prat A, Antel J, Bruck W, Lassmann H (2017) An updated histological classification system for multiple sclerosis lesions. Acta Neuropathol 133:13–24. https://doi.org/10.1007/s00401-016-1653-y
39. Kuhlmann T, Miron V, Cui Q, Wegner C, Antel J, Brück W (2008) Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131:1749–1758
40. Kuhlmann T, Remington L, Maruschak B, Owens T, Bruck W (2007) Nogo-A is a reliable oligodendroglial marker in adult human and mouse CNS and in demyelinated lesions. J Neuropathol Exp Neurol 66:238–246
41. Lambert C, Ase AR, Seguela P, Antel JP (2010) Distinct migratory and cytokine responses of human microglia and macrophages to ATP. Brain Behav Immun 24:1241–1248. https://doi.org/10.1016/j.bbi.2010.02.010
42. Lloyd AF, Miron VE (2019) The pro-remyelination properties of microglia in the central nervous system. Nat Rev Neurol 15:447–458. https://doi.org/10.1038/s41582-019-0184-2
43. Locatelli G, Theodorou D, Kendirli A, Jordao MJC, Staszewski O, Phulphagar K et al (2018) Mononuclear phagocytes locally

specify and adapt their phenotype in a multiple sclerosis model. Nat Neurosci 21:1196–1208. https://doi.org/10.1038/s41593-018-0212-3
44. Lombardi M, Parolisi R, Scaroni F, Bonfanti E, Gualerzi A, Gabrielli M et al (2019) Detrimental and protective action of microglial extracellular vesicles on myelin lesions: astrocyte involvement in remyelination failure. Acta Neuropathol 138:987–1012. https://doi.org/10.1007/s00401-019-02049-1
45. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 47:707–717
46. Luchetti S, Fransen NL, van Eden CG, Ramaglia V, Mason M, Huitinga I (2018) Progressive multiple sclerosis patients show substantial lesion activity that correlates with clinical disease severity and sex: a retrospective autopsy cohort analysis. Acta Neuropathol 135:511–528. https://doi.org/10.1007/s00401-018-1818-y
47. Manrique-Hoyos N, Jurgens T, Gronborg M, Kreutzfeldt M, Schedensack M, Kuhlmann T et al (2012) Late motor decline after accomplished remyelination: impact for progressive multiple sclerosis. Ann Neurol 71:227–244
48. Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcao A, Xiao L et al (2016) Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. Science 352:1326–1329. https://doi.org/10.1126/science.aaf6463
49. Matsushima GK, Morell P (2001) The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 11:107–116
50. Mei F, Fancy SP, Shen YA, Niu J, Zhao C, Presley B et al (2014) Micropillar arrays as a high-throughput screening platform for therapeutics in multiple sclerosis. Nat Med 20:954–960
51. Mi S, Miller RH, Tang W, Lee X, Hu B, Wu W et al (2009) Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. Ann Neurol 65:304–315
52. Miron VE, Boyd A, Zhao JW, Yuen TJ, Ruckh JM, Shadrach JL et al (2013) M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci 16:1211–1218
53. Najm FJ, Madhavan M, Zaremba A, Shick E, Karl RT, Factor DC et al (2015) Drug-based modulation of endogenous stem cells promotes functional remyelination in vivo. Nature 522:216–220
54. Nave KA, Ehrenreich H (2019) Time to revisit oligodendrocytes in multiple sclerosis. Nat Med 25:364–366. https://doi.org/10.1038/s41591-019-0388-4
55. Ortiz FC, Habermacher C, Graciarena M, Houry PY, Nishiyama A, Nait Oumesmar B et al (2019) Neuronal activity in vivo enhances functional myelin repair. JCI Insight. https://doi.org/10.1172/jci.insight.123434
56. Patani R, Balaratnam M, Vora A, Reynolds R (2007) Remyelination can be extensive in multiple sclerosis despite a long disease course. Neuropathol Appl Neurobiol 33:277–287
57. Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidtbauer M, Laursen H et al (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129:3165–3172
58. Pennati A, Nylen EA, Duncan ID, Galipeau J (2020) Regulatory B cells normalize CNS myeloid cell content in a mouse model of multiple sclerosis and promote oligodendrogenesis and remyelination. J Neurosci. https://doi.org/10.1523/JNEUROSCI.2840-19.2020
59. Pittock SJ, McClelland RL, Achenbach SJ, Konig F, Bitsch A, Bruck W et al (2005) Clinical course, pathological correlations, and outcome of biopsy proved inflammatory demyelinating disease. J Neurol Neurosurg Psychiatry 76:1693–1697. https://doi.org/10.1136/jnnp.2004.060624
60. Reinhardt P, Glatza M, Hemmer K, Tsytsyura Y, Thiel CS, Hoing S et al (2013) Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling. PLoS ONE 8:e59252
61. Segel M, Neumann B, Hill MFE, Weber IP, Viscomi C, Zhao C et al (2019) Niche stiffness underlies the ageing of central nervous system progenitor cells. Nature 573:130–134. https://doi.org/10.1038/s41586-019-1484-9
62. Stangel M, Kuhlmann T, Matthews PM, Kilpatrick TJ (2017) Achievements and obstacles of remyelinating therapies in multiple sclerosis. Nat Rev Neurol 13:742–754. https://doi.org/10.1038/nrneurol.2017.139
63. Tallantyre EC, Bo L, Al-Rawashdeh O, Owens T, Polman CH, Lowe JS et al (2010) Clinico-pathological evidence that axonal loss underlies disability in progressive multiple sclerosis. Mult Scler 16:406–411
64. Targett M, Sussman J, Scolding N, O'Leary MT, Compston D, Blakemore WF (1996) Failure to achieve remyelination of demyelinated rat axons following transplantation of glial cells obtained from the adult human brain. Neuropathol Appl Neurobiol 22:199–206
65. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338:278–285
66. Wake H, Ortiz FC, Woo DH, Lee PR, Angulo MC, Fields RD (2015) Nonsynaptic junctions on myelinating glia promote preferential myelination of electrically active axons. Nat Commun 6:7844. https://doi.org/10.1038/ncomms8844
67. Weider M, Starost LJ, Groll K, Kuspert M, Sock E, Wedel M et al (2018) Nfat/calcineurin signaling promotes oligodendrocyte differentiation and myelination by transcription factor network tuning. Nat Commun 9:899. https://doi.org/10.1038/s41467-018-03336-3
68. Wolswijk G (1998) Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. J Neurosci 18:601–609
69. Wolswijk G (2002) Oligodendrocyte precursor cells in the demyelinated spinal cord. Brain 125:338–349
70. Wolswijk G (1998) Oligodendrocyte regeneration in the adult rodent CNS and the failure of this process in multiple sclerosis. Prog Brain Res 117:233–247
71. Yeung M, Djelloul M, Steiner E, Bernard S, Salehpour M, Possnert G et al (2019) Dynamics of oligodendrocyte generation in multiple sclerosis. Nature 566:538–542. https://doi.org/10.1038/s41586-018-0842-3
72. Zhang Y, Argaw AT, Gurfein BT, Zameer A, Snyder BJ, Ge C et al (2009) Notch1 signaling plays a role in regulating precursor differentiation during CNS remyelination. Proc Natl Acad Sci USA 106:19162–19167