BRAIN
ORIGINAL ARTICLE

BCAS1-positive oligodendrocytes enable efficient cortical remyelination in multiple sclerosis

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Remyelination is a crucial regenerative process in demyelinating diseases, limiting persisting damage to the CNS. It restores saltatory nerve conduction and ensures trophic support of axons. In patients with multiple sclerosis, remyelination has been observed in both white and grey matter and found to be more efficient in the cortex. Brain-enriched myelin-associated protein 1 (BCAS1) identifies oligodendrocyte lineage cells in the stage of active myelin formation in development and regeneration. Other than in the white matter, BCAS1+ oligodendrocytes are maintained at high densities in the cortex throughout life.

Here, we investigated cortical lesions in human biopsy and autopsy tissue from patients with multiple sclerosis in direct comparison to demyelinating mouse models and demonstrate that following a demyelinating insult BCAS1+ oligodendrocytes in remyelinating cortical lesions shift from a quiescent to an activated, internode-forming morphology co-expressing myelin-associated glycoprotein (MAG), necessary for axonal contact formation. Of note, activated BCAS1+ oligodendrocytes are found at early time points of experimental demyelination amidst ongoing inflammation. In human tissue, activated BCAS1+ oligodendrocytes correlate with the density of myeloid cells, further supporting their involvement in an immediate regenerative response.

Furthermore, studying the microscopically normal appearing non demyelinated cortex in patients with chronic multiple sclerosis, we find a shift from quiescent BCAS1+ oligodendrocytes to mature, myelin-maintaining oligodendrocytes, suggesting oligodendrocyte differentiation and limited replenishment of BCAS1+ oligodendrocytes in long-standing disease. We also demonstrate that part of perineuronal satellite oligodendrocytes are BCAS1+ and contribute to remyelination in human and experimental cortical demyelination.

In summary, our results provide evidence from human tissue and experimental models that BCAS1+ cells in the adult cortex represent a population of pre-differentiated oligodendrocytes that rapidly react after a demyelinating insult thus enabling immediate myelin regeneration. In addition, our data suggest that limited replenishment of BCAS1+ oligodendrocytes may contribute to the remyelination failure observed in the cortex in chronic multiple sclerosis.

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Introduction

Remyelination is a regenerative process of the adult CNS, whereby saltatory impulse conduction and trophic support to axons are restored.[1-5] It is initiated after internode loss as occurs in demyelinating diseases such as multiple sclerosis (MS).[6] The efficacy of remyelination has been shown to depend on multiple factors such as, age, disease duration and lesion location.[7-14] Several neuropathological reports indicate that the cerebral cortex in MS remyelinates more efficiently compared to white matter lesions in the same patient, and that differences in extracellular matrix and cellular composition may contribute.[15-17] Recently, MRI studies in people with MS found an inverse correlation of cortical remyelination and cortical atrophy, and an effect on disease progression in a subgroup with less demyelination and shorter disease duration.[18]

The origin of remyelinating oligodendrocytes in MS and demyelinating animal models has recently again become subject to discussion. The assumption that surviving adult oligodendrocytes contribute to remyelination has received novel support by findings in a cat and a B12-deficient non-human primate model as well as single cell analyses of oligodendrocytes in human brain tissue from patients with MS.[19-21] In addition, birth dating of oligodendrocytes by analysis of integrated nuclear bomb test-derived ^{14}C revealed that few oligodendrocytes were newly generated in shadow plaques of MS patients, supporting the notion that adult oligodendrocytes are capable of generating new myelin sheaths after demyelination.[22] Importantly, however, a multitude of experimental evidence has shown that remyelination is associated with proliferation and differentiation of oligodendrocyte precursor cells into mature oligodendrocytes,[23-25] and it has been demonstrated that newly formed cells might be much more efficient compared to surviving oligodendrocytes.[26-28] These findings promoted a debate on the origins of new internodes that might derive from different cellular sources depending on the nature, extent, duration and potentially age of the demyelinating insult.

We and others have recently shown that immunohistochemistry for brain-enriched myelin-associated protein 1 (BCAS1) identifies process-rich pre-myelinating and internode-forming actively myelinating oligodendrocytes in mouse and human tissue.[29-31] BCAS1+ oligodendrocytes are Olig2+ but do not express markers of oligodendrocyte precursor cells (e.g. NG2) and mature oligodendrocytes (e.g. CC1 or p25/TTTP), thus representing an intermediate stage of oligodendrocyte differentiation. BCAS1+ oligodendrocytes are highly abundant in the white matter during developmental myelination but largely disappear in adulthood. In contrast, in the cerebral cortex, the population of BCAS1+ oligodendrocytes remains substantial throughout life and only slowly decreases with age.[29]

In this study, we hypothesized that BCAS1+ oligodendrocytes in the cerebral cortex may represent a population of pre-differentiated oligodendrocytes contributing to fast remyelination of cortical demyelinated areas and the heightened repair efficiency of cortical grey matter previously reported. We thus investigated the response of cortical BCAS1+ oligodendrocytes to demyelination in early and late stage MS and cuprizone- as well as antibody-induced targeted cortical demyelination.

Materials and methods

Human tissue

Formalin-fixed, paraffin-embedded brain tissue from archival biopsy and autopsy cases was obtained from the Institute of Neuropathology at the University Medical Center Göttingen and the MS Brain Bank of the Federal Ministry of Science (BMBF)-funded disease-oriented Competence Network Multiple Sclerosis (KKNMS). We studied 24 biopsies and 26 autopsies of patients with MS (Supplementary Tables 1 and 2) and 11 control biopsies (age range 33 to 85 years; Supplementary Table 3) and 31 control autopsies (age range 18–89 years; Supplementary Table 4). MS biopsies were performed for diagnostic reasons to exclude tumour or infection and targeted MRI-defined white matter lesions (Supplementary Table 1). Biopsy and autopsy tissues were classified according to demyelinating lesion activity as described previously[32] and assessed with regard to the presence of CD3+ T cells in cortex and meninges (Supplementary Tables 5 and 6 and Supplementary Figs 2 and 3). Demyelinated, remyelinated and normal appearing cortex areas were identified after analysis of (immuno-)histochemical stainings for multiple myelin components (Supplementary Fig. 4). Control biopsies contained microscopically inconspicuous white matter and cortex with, if at all,

only minimal areas of diseased tissue. Control autopsies showed no CNS pathology. The median age of MS patients at biopsy was 40 years (interquartile range, 32–49 years); the median time from first symptoms to biopsy was 3 months (interquartile range, 0.5–12 years; patients with unknown disease duration were excluded); and the female to male ratio was 2:1. The clinical diagnosis at the time of biopsy was clinically isolated syndrome (CIS) in 12, relapsing-remitting MS (RRMS) in eight and secondary progressive MS (SPMS)/primary progressive MS (PPMS) in four cases. The ages of the 26 autopsied MS patients ranged from 38 to 74 years (median 57, interquartile range, 46–63 years), with a female to male ratio of 1:1.25. All patients, except Patient 7 (disease duration: 12 years), Patient 9 (>4 years), Patient 14 (12 years) and Patient 26 (14 years), had a disease duration of >15 years and showed a progressive disease course. The study was approved by the local ethics committee.

Mice

Six- to seven-week-old C57BL/6J mice were purchased from Charles River (maximum six animals per cage). Th/+ mice (MOG-specific Ig heavy-chain knock-in mice, kindly provided by Tobias Litzenburger, Antonio Iglesias and Hartmut Wekerle) were bred at the animal facility of the University Medical Center Göttingen under specific-pathogen-free conditions.[33] All animals were housed under a 12/12 h light/dark cycle and had access to food and water *ad libitum*.

Cuprizone-induced demyelination

For cuprizone-induced demyelination experiments, 7- to 8-week-old male C57BL/6J mice were fed for either 1, 2, 3, 4 or 6 weeks with cuprizone [Bis(cyclohexanone)oxaldihydrazone, 0.25% in normal chow]. Mice were sacrificed 1, 2, 3, 4 and 6 weeks after initiation of the cuprizone diet to assess demyelination. Mice were sacrificed 2, 4, 6 and 21 days after cuprizone diet cessation to analyse remyelination. Mice were perfusion-fixed with 4% paraformaldehyde (PFA), and brains were embedded into paraffin and processed for histochemistry and immunohistochemistry.

Induction of focal cortical demyelination in Th/+ mice

Th/+ mice were immunized subcutaneously with 100 μg recombinant rat myelin oligodendrocyte glycoprotein (MOG)1–125/CFA. Three hundred nanograms of pertussis toxin (List Biological Laboratories, Cat. No. 180) was injected intraperitoneally at Days 0 and 2 after immunization.[34] Experimental autoimmune encephalitis (EAE) animals were scored as previously described.[35] For stereotactic lesion induction, anaesthetized rMOG immunized Th/+ mice received a stereotactic injection on the 2nd day of disease. Two microlitres of a cytokine mixture composed of 50 ng TNFα (R&D Systems) and 60 ng IFNγ (R&D Systems) dissolved in PBS was injected together with Monastral blue (Fluka) to facilitate the identification of the lesions in the tissue (0.1 mm caudal to the bregma, 0.2 mm lateral to the midline, 0.7 mm depth), according to protocols described previously.[36]

Statistical analysis

All statistics were calculated using the GraphPad Prism 6 (GraphPad Software) and SPSS version 27.0 Software. The Kolmogorov–Smirnov test with the Dallal–Wilkinson–Lillie test for the *P*-value were carried out to test for normal distribution. To compare two groups, a Student's two-tailed *t*-test or the Mann–Whitney test was applied. One-way ANOVA followed by Bonferroni's or Tukey's *post hoc* test or one-way ANOVA with Kruskal–Wallis test with Dunn's multiple comparison test was performed for more than two groups. Alternatively, two-way ANOVA with Sidak's multiple comparison test was used. For the correlation of interval-scaled clinical features with cell densities, Spearman's rho correlation analysis was performed. Data are mean ± standard error of the mean if not stated otherwise.

Results

Increased activated BCAS1+ oligodendrocytes in the cortex at the peak of experimental demyelination

BCAS1+ oligodendrocytes in the cortex appear in two main phenotypes: cells with few or no immunopositive processes, prevailing in the healthy adult brain ('resting' BCAS1+ oligodendrocytes; arrows in Fig. 1A) and cells with a pre-myelinating morphology with multiple branches and myelinating morphology, characterized by internode formation ('activated' BCAS1+ oligodendrocytes; arrowheads in Fig. 1A).[31,37,38]

We have shown previously that BCAS1+ oligodendrocyte density is increased in rodent white matter during myelin regeneration after toxic demyelination.[29] Here, we assessed whether BCAS1 is also useful to detect ongoing remyelination in the mouse cortex. We exposed mice to the demyelinating agent cuprizone for 6 weeks and quantified the density of all BCAS1+ oligodendrocytes in the cortex at multiple time points during treatment and after removal of cuprizone from the diet. We found that the total number of BCAS1+ cells in the cortex did not change significantly during treatment and after discontinuation of cuprizone (Fig. 1A and B). However, when we specifically quantified BCAS1+ oligodendrocytes with activated morphology, we saw a clear increase in myelinating cells compared to naïve mice from the very beginning of the cuprizone diet (though not significant in the first week; Fig. 1C). This increase reached its maximum at a time point when the loss of mature tubulin polimerization promoting protein (TPPP/p25)+ oligodendrocytes was severe and still ongoing in the cortex (Week 4; Fig. 1D).

We then investigated whether this early increase in activated BCAS1+ oligodendrocytes, found in the cortex under conditions of toxic oligodendrocyte damage, is a general feature of cortical demyelinating lesions and can also be observed in an animal model of antibody-mediated demyelination. To this end, we induced EAE in Th/+ mice harbouring anti-MOG antibodies in their sera by immunization with recombinant $MOG^{1–125}$ protein. At disease onset, a mixture of cytokines (50 ng TNFα and 60 ng IFNγ) was stereotactically injected into the cortex, thus eliciting a focal inflammatory demyelinating lesion.[34,36] We quantified actively myelinating BCAS1+ oligodendrocytes within the lesion at different time points after induction and related them to the presence of macrophages/activated microglia. We found that activated myelinating BCAS1+ cells were highest soon after the demyelinating insult, as early as 5 days after lesion induction (Fig. 1E and G), when high numbers of infiltrating Mac3+ phagocytes were still present in the lesion (Fig. 1H). Importantly, relying on markers of compact myelin such as MBP, the expansion of the demyelinated area at this early stage of lesion formation was at its maximum, and no evidence for newly formed MBP+ internodes was found yet (Fig. 1E and I).

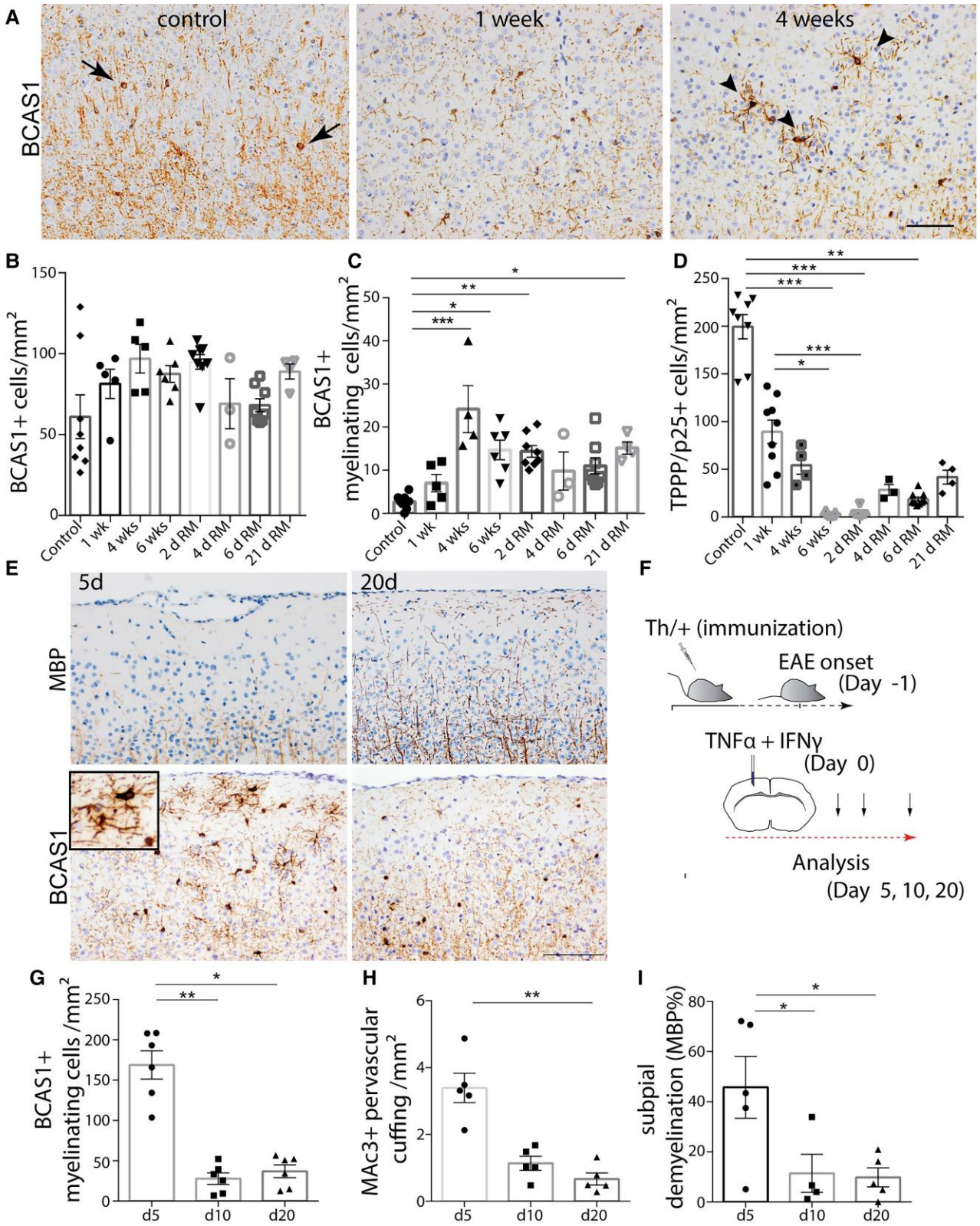

Figure 1 BCAS1+ myelinating oligodendrocytes are generated rapidly after toxic and inflammatory cortical lesion induction. (**A**) BCAS1+ oligodendrocytes in the cerebral cortex of control and cuprizone-treated mice. In control cortex, BCAS1+ oligodendrocytes with no or few processes predominate (*left*, arrows), whereas after 4 weeks of cuprizone treatment, BCAS1+ oligodendrocytes adopt a myelinating, process-rich phenotype (*right*, arrow heads). (**B**) BCAS1+ oligodendrocyte density in the cerebral cortex of control mice during cuprizone treatment and after cuprizone withdrawal. (**C**) Quantification of BCAS1+ process-rich myelinating cells in the cerebral cortex of control mice during cuprizone treatment and after cuprizone withdrawal [control, $n = 8$; 1 week, $n = 5$; 4 weeks, $n = 5$; 6 weeks, $n = 6$; 2 days remyelination (2 d RM), $n = 8$; 4 days RM, $n = 3$; 6 days RM, $n = 8$; 21 days RM, $n = 4$;

(Continued)

BCAS1+ satellite oligodendrocytes participate in remyelination

In the grey matter, a subpopulation of oligodendrocytes in close proximity to neurons is designated perineuronal satellite cells.[39] Whether these satellite cells can participate in myelin regeneration or are primarily engaged in local neuronal support is not resolved. We confirmed that about one-third of BCAS1+ oligodendrocytes in control mouse cortex were satellite cells (37.4 ± 2.5%). We then asked whether these BCAS1+ satellite oligodendrocytes participate in remyelination at specific time points of cuprizone treatment. Quantification revealed that BCAS1+ satellite cells with an activated morphology were increased during toxic demyelination compared to controls, with a peak at 4 weeks under treatment (Fig. 2B). The percentage of BCAS1+ satellite cells co-expressing cytosolic MAG, which is essential for early oligodendrocyte–axon contact, showed a tendency to increase from Week 4 under treatment, further indicating ongoing formation of novel myelin sheaths (not significant; Fig. 2C). While only a subpopulation of BCAS1+ oligodendrocytes co-expressed MAG, practically all oligodendrocytes with MAG+ processes were BCAS1+ (data not shown). Judging from cellular morphology, MAG was expressed at a slightly later stage of oligodendrocyte differentiation in cells with newly forming internodes. BCAS1+ as well as cytosolic and process-based MAG expression was lost in mature myelin-maintaining TPPP/p25+ cells (Figs 2A and 3E). No change in the total number of satellite oligodendrocytes, of BCAS1+ cells or the percentage of BCAS1+ satellite cells was observed during cuprizone treatment (data not shown).

Activated BCAS1+ oligodendrocytes are increased in early demyelinating human cortical lesions

Our experimental data suggested that activated BCAS1+ oligodendrocytes in the cortex are generated early after lesion initiation and amidst ongoing toxic oligodendrocyte loss (cuprizone) and myelin phagocytosis (antibody-induced demyelination). To test whether a similar early response to demyelination can be observed in human cortical demyelination, we assessed grey matter lesions obtained from brain biopsies of patients with MS early in the disease course[32,40] for the presence of BCAS1+ oligodendrocytes.

About two thirds of the examined cortical lesions in MS biopsy tissue displayed signs of early ongoing remyelination, i.e. an abundance of BCAS1+ process-bearing oligodendrocytes in the absence of MBP+ fibres (remyelinating lesions, 15/24 lesions) (Fig. 3A and B). Similar to what we found in the cuprizone model, the total number of BCAS1+ cells in remyelinating lesions was comparable to control tissue (normal cortex) and normal-appearing cortex of MS patients (defined by microscopically normal myelination assessed in MBP-immunohistochemistry; Fig. 3C). In contrast, the total number of BCAS1+ oligodendrocytes was severely reduced in demyelinated lesions (Fig. 3A and C). Similar densities of activated microglia/macrophages (KiM1P+) were observed in early demyelinated and early remyelinating lesions (Fig. 3A and D), further underlining their close temporal relationship and the rapid activation of BCAS1+ oligodendrocytes after demyelination. To detect a possible influence of age, sex, disease type or disease duration on the density of BCAS1+ pre-myelinating and actively myelinating oligodendrocytes in MS biopsies, Spearman's rho correlation (age, disease duration) and the Mann–Whitney U-test (sex, disease type) were performed. However, no robust correlations were detected, and a larger sample size is required to properly determine the influence of these parameters.

Increased numbers of activated BCAS1+ oligodendrocytes in remyelinating cortical areas of chronic MS

Having established BCAS1 as a reliable marker for active remyelination in the cortex of patients in early stages of MS, we next asked whether evidence for ongoing remyelination was also observed in cortical lesions of patients with long-standing disease. Investigating autopsy tissue from 26 patients with chronic MS, we identified increased numbers of activated BCAS1+ oligodendrocytes in 26.1% of the cortical lesions studied (Fig. 4A and B), evidencing ongoing remyelination (see also Supplementary Fig. 1 for axonal contact formation by BCAS1+ oligodendrocytes). Similar to the observations made in biopsy tissue, the total number of BCAS1+ oligodendrocytes in these remyelinating areas was not changed compared to control tissue, while it was clearly reduced in fully demyelinated, non-remyelinating cortical lesions (Fig. 4C).

A shift from BCAS1+ to mature oligodendrocytes in the normal-appearing cortex of patients with chronic MS

Our data so far indicated that successful cortical remyelination occurs in areas with normal densities of BCAS1+ oligodendrocytes, from which internode-forming, activated BCAS1+ cells can be recruited. As BCAS1+ cells represent a transitional stage of oligodendrocyte differentiation between oligodendrocyte progenitor cells and myelin-maintaining oligodendrocytes, their differentiation fills up or adds to the population of TPPP/p25 mature oligodendrocytes. Thus, previous differentiation stimuli, e.g. demyelination and inflammation, might lead to a reduction in BCAS1+ oligodendrocytes in the cortex, if they are not sufficiently replenished by the proliferation and differentiation of oligodendrocyte precursor cells. To investigate whether such a shift in cortical oligodendrocyte populations occurs in chronic MS, we quantified the density of BCAS1 and TPPP/p25+ cells in normal cortex, the microscopically regularly myelinated normal-appearing cortex and cortical lesions showing demyelination and remyelination. Importantly and in contrast to our findings in early MS (Fig. 3), the total number of BCAS1+ oligodendrocytes was significantly reduced in the normal-appearing cortex of patients with chronic MS compared to the age-matched normal cortex (Fig. 4A and C). In addition, the density of TPPP/p25+ mature, myelin-maintaining oligodendrocytes was

Figure 1 Continued
Kruskal–Wallis test with Dunn's multiple comparison test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; mean ± standard error of the mean]. (**D**) Density of TPPP/p25+ myelin-maintaining oligodendrocytes in the cerebral cortex during de- and remyelination (controls, $n = 8$; 1 week, $n = 9$; 4 weeks, $n = 4$; 6 weeks, $n = 5$; 2 days RM, $n = 8$; 4 days RM, $n = 3$; 6 days RM, $n = 9$; 21 days RM, $n = 4$; Kruskal–Wallis test with Dunn's multiple comparison test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). (**E**) MBP and BCAS1 immunohistochemistry (IHC) on Days 5 and 20 after focal lesion induction in a model of antibody-induced cortical demyelination (schematic representation in **F**). (**G**) BCAS1+ myelinating oligodendrocytes (Days 5, 10 and 20; $n = 6$; Kruskal–Wallis test with Dunn's *post hoc* test; *$P < 0.05$, **$P < 0.01$). (**H**) Quantification of perivascular Mac3+ infiltrating macrophages/activated microglia (Days 5, 10 and 20; $n = 5$; Kruskal–Wallis test with Dunn's *post hoc* test; **$P < 0.01$). (**I**) Percentage of demyelination in subpial cortical area, assessed by anti-MBP IHC (Days 5, $n = 5$; 10, $n = 4$; and 20, $n = 5$; Kruskal–Wallis test with Dunn's *post hoc* test; *$P < 0.05$). Scale bars = 100 µm. EAE = experimental autoimmune encephalitis.

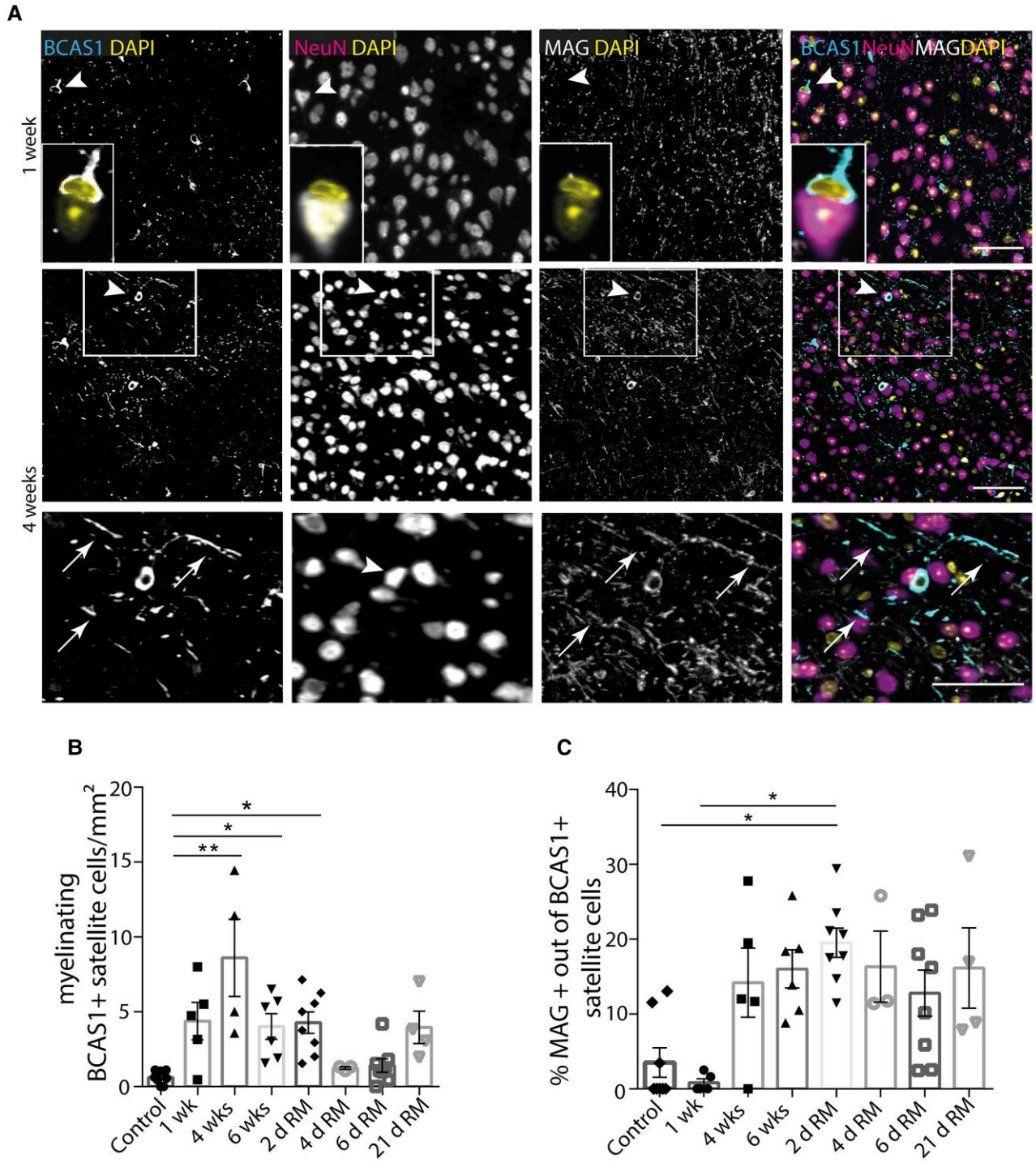

Figure 2 BCAS1+ perineuronal satellite oligodendrocytes contribute to remyelination in cuprizone-induced demyelination. (**A**) Immunofluorescence triple labelling of BCAS1 (cyan) with NeuN (neuronal nuclear antigen, magenta) and the myelin-associated glycoprotein (MAG, white). *Insets*: Magnification of the BCAS1+ satellite oligodendrocytes as indicated by the arrowhead. *Bottom*: Magnification of internode-forming BCAS1+ oligodendrocytes forming parallel contacts with multiple neighbouring axons. (**B**) Density of BCAS1+ satellite oligodendrocytes with a process-rich myelinating morphology in control mice, during cuprizone-induced demyelination and after cuprizone withdrawal [controls, $n = 8$; 1 week, $n = 5$; 4 weeks, $n = 4$; 6 weeks, $n = 6$; 2 days remyelination (2 d RM), $n = 8$; 4 days RM, $n = 3$; 6 days RM, $n = 8$; 21 days RM, $n = 4$; Kruskal-Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$, $^{**}P < 0.01$]. (**C**) Percentage of BCAS1+ perineuronal satellite oligodendrocytes that express MAG during cuprizone-induced demyelination and after cuprizone withdrawal (controls, $n = 8$; 1 week, $n = 5$; 4 weeks, $n = 5$; 6 weeks, $n = 6$; 2 days RM, $n = 8$; 4 days RM, $n = 3$; 6 days RM, $n = 8$; 21 days RM, $n = 4$; Kruskal–Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$). Scale bars = 50 µm.

significantly increased in the normal-appearing cortex compared to remyelinating cortical lesions and normal cortex (Fig. 4E and F), while the total number of Olig2+ cells, representing all cells of the oligodendrocyte lineage, was found to be unaltered (Fig. 4E and G).

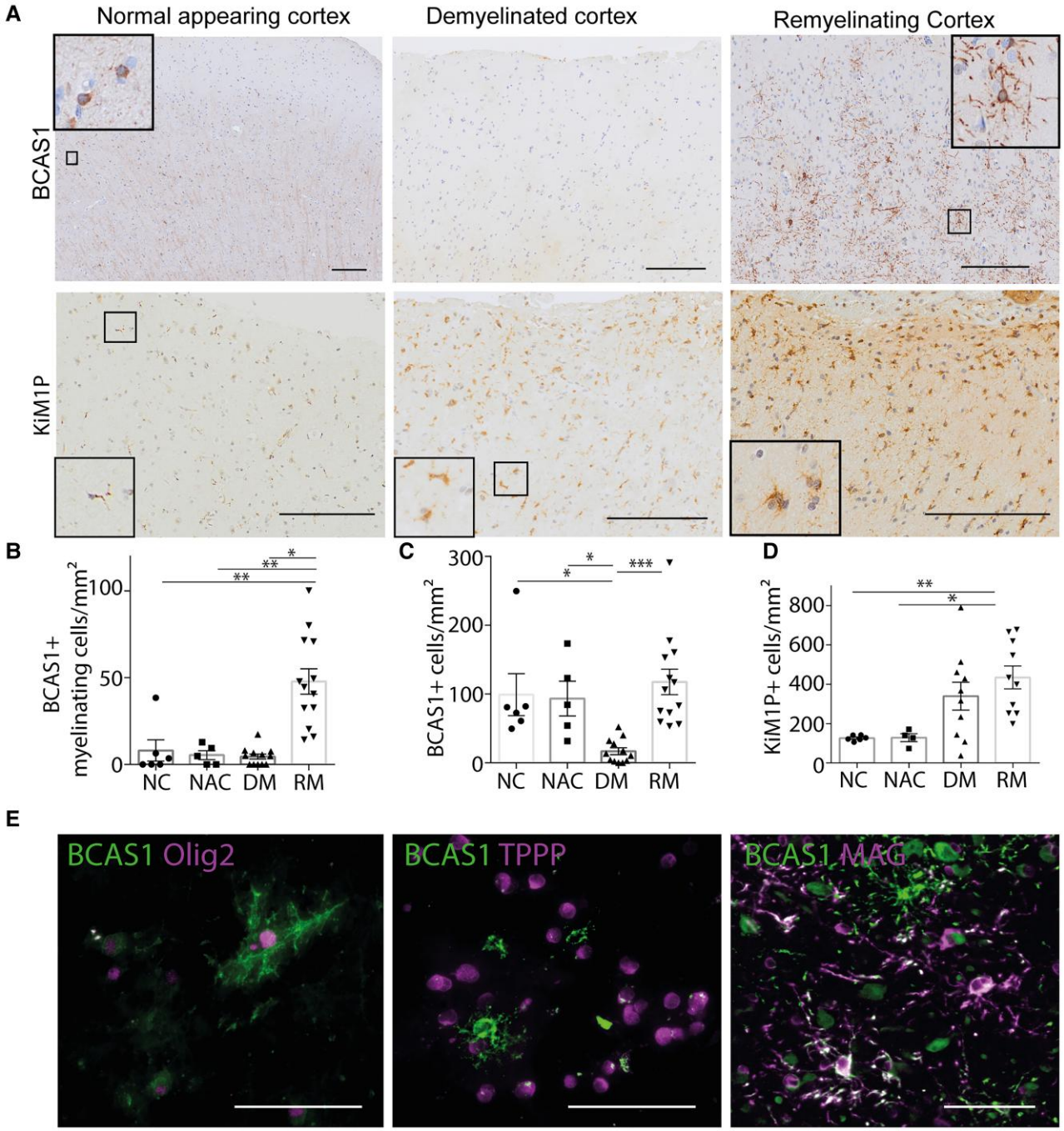

Figure 3 Activation of BCAS1+ oligodendrocytes in remyelinating cortical biopsy tissue of early multiple sclerosis. (**A**) BCAS1 and KiM1P (macrophages/activated microglia) immunohistochemistry (IHC) of cerebral cortex of biopsied multiple sclerosis (MS) patients containing normal-appearing cortex (NAC), demyelinated (DM) and remyelinating (RM) cortical lesions. In the NAC, BCAS1+ oligodendrocytes show a round, perinuclear staining and no branches. Phagocytes show a homeostatic morphology and are weakly stained by KiM1P (*left*). DM areas show a reduction in BCAS1+ oligodendrocytes among activated Kim1P+ phagocytes (*middle*). Within remyelinating areas, branched, internode-forming BCAS1+ oligodendrocytes are observed, and phagocytes display an activated morphology with increased KiM1P staining (*right*). (**B**) Density of BCAS1+ oligodendrocytes with a process-rich myelinating morphology in the cortex of MS patients (NC, $n = 6$; NA $n = 5$; DM, $n = 12$; RM, $n = 13$; Kruskal–Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$, $^{**}P < 0.01$). (**C**) Density of all BCAS1+ oligodendrocytes in the cortex of MS patients (NC, $n = 6$; NAC, $n = 5$; DM, $n = 12$; RM, $n = 13$; Kruskal–Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$, $^{***}P < 0.001$). (**D**) Quantification of KiM1P+ macrophages/activated microglia cells in the cortex of MS patients (NC, $n = 6$; NAC, $n = 4$; DM, $n = 10$; RM, $n = 10$; Kruskal–Wallis test with Dunn's *post hoc* test; $^{*}P < 0.05$, $^{**}P < 0.01$). (**E**) Co-labelling of BCAS1 and oligodendrocyte lineage marker in MS biopsy tissue. Error bars indicate mean ± standard error of the mean. Scale bars in **A** = 200 µm and **E** = 50 µm.

This may indicate a shift from BCAS1+ to TPPP/p25+ oligodendrocytes in the normal-appearing cortex of long-standing MS and hint towards a chronic exposure to differentiating stimuli, most likely associated with chronic inflammation. In contrast, in chronic demyelinated cortical lesions, we observed a reduction in all oligodendrocyte lineage cells (Olig2), including BCAS1+ and TPPP/p25+

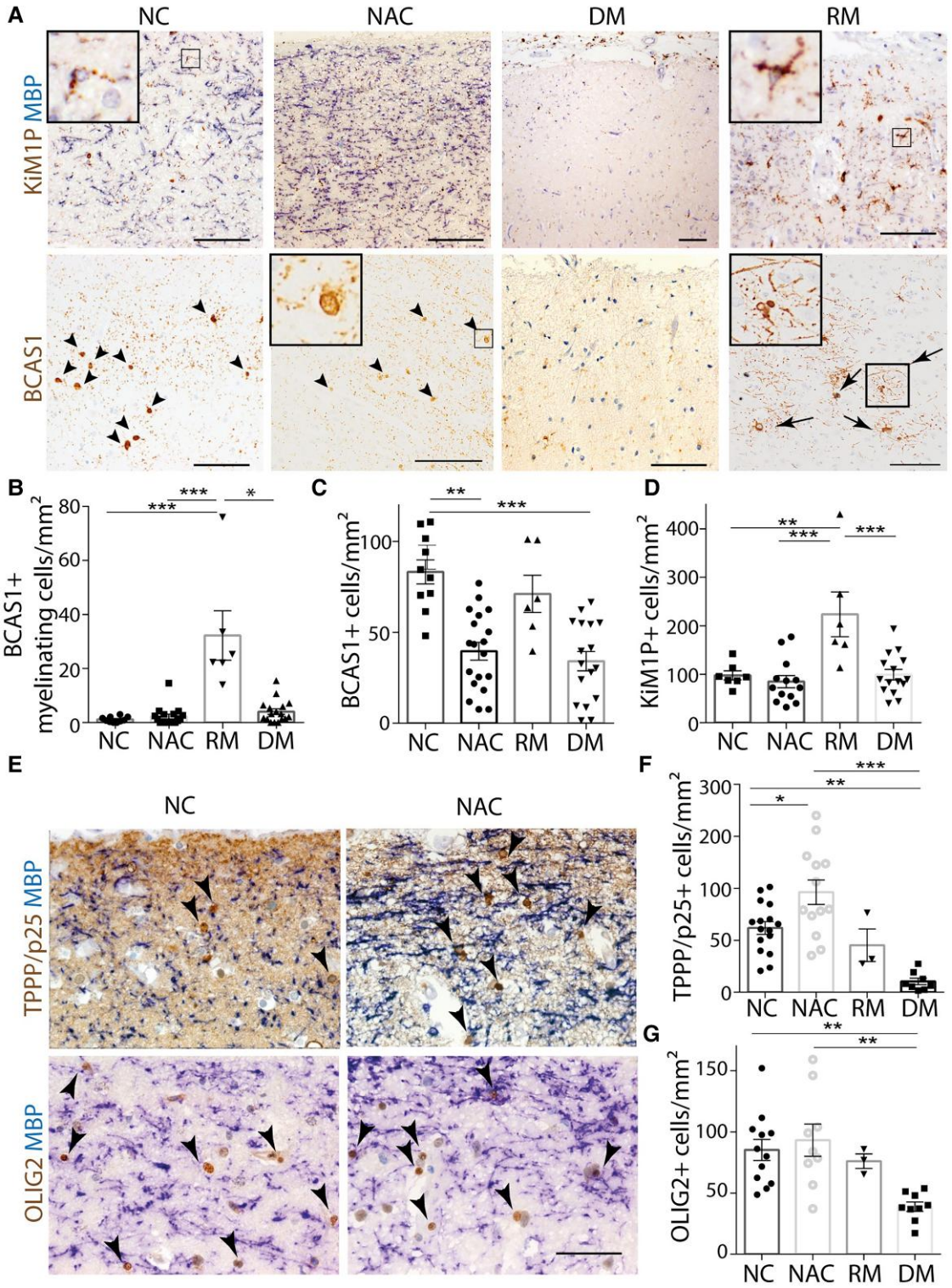

Figure 4 BCAS1+ oligodendrocytes are decreased, while myelin-maintaining oligodendrocytes are increased in the normal-appearing cortex in patients with progressive multiple sclerosis. (**A**) Co-labelling of KiM1P (macrophages/activated microglia) with MBP and BCAS1 of cerebral cortex of multiple sclerosis (MS) patients containing normal-appearing cortex (NAC), demyelinated (DM) and remyelinating (RM) cortical lesions and normal cortex (NC) of age-matched controls. Arrowheads indicate BCAS1+ oligodendrocytes in NC and NAC, while arrows indicate process-rich actively myelinating BCAS1+ oligodendrocytes in remyelinating areas, magnified in *insets* for NAC and RM areas. In addition, homeostatic versus activated morphology of KiM1P+ phagocytes is shown in higher magnification for NC and RM. (**B**) Density of BCAS1+ oligodendrocytes with a myelinating morphology in the cortex of MS patients and age-matched controls (NC, $n = 10$; NAC, $n = 17$; DM, $n = 17$; RM, $n = 6$; Kruskal-Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$, $^{***}P < 0.001$). (**C**) Total density of BCAS1+ oligodendrocytes in the cortex of MS patients and age-matched controls (NC, $n = 10$; NAC, $n = 19$; DM, $n = 17$; RM, $n = 6$; Kruskal–Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). (**D**) Density of KiM1P+ macrophages/activated microglia in the cortex of MS patients and age-matched controls (NC, $n = 7$; NAC, $n = 13$; DM, $n = 15$; RM, $n = 6$; one-way ANOVA

(Continued)

cells, suggesting an overall consumption of oligodendrocytes, as reported previously.[41] Of note, and similar to early cortical lesions in biopsies, autoptic cortical lesions with ongoing remyelination contained significantly higher numbers of activated microglia/macrophages (Fig. 4D). To determine any significant effect of age, sex, disease type or disease duration on oligodendroglia densities in our MS autopsy cohort, we performed Spearman's rho correlation analyses for age and disease duration, and Mann–Whitney U-test of subgroups separated by sex and diagnosis but could not ascertain any significant correlations. More samples might be required to finally determine the impact of clinical parameters on BCAS1+ pre-myelinating and actively myelinating oligodendrocyte densities in MS autopsy tissue.

Satellite cells in human cortical lesions are in part BCAS1+ and contribute to remyelination

We have shown that BCAS1+ satellite oligodendrocytes contribute to remyelination in cuprizone-induced cortical demyelination. To study whether satellite oligodendrocytes also take part in myelin regeneration in MS, we first confirmed that a proportion of BCAS1 + cells in the human cortex classify as satellite cells (40.1 ± 4.8% in control biopsy tissue). We then quantified BCAS1+ satellite oligodendrocytes with a myelinating morphology and BCAS1+ satellite oligodendrocytes co-expressing MAG in biopsy tissue of cortical MS lesions. Both methods revealed that activated BCAS1+ satellite oligodendrocytes were significantly increased in early remyelinating cortical lesions compared to control cortex (Fig. 5A–D). These results demonstrated that BCAS1+ perineuronal satellite oligodendrocytes take an active part in the remyelination of cortical lesions in patients with MS. Next, we tested whether the above demonstrated shift towards mature oligodendrocyte populations in the normal appearing cortex in chronic MS could also be seen specifically in satellite oligodendrocytes. Indeed, we found a significant increase in perineuronally localized TPPP/p25+ satellite cells in the normal appearing cortex in autopsy tissue of patients with MS compared to age-matched controls (Fig. 5E and F).

Discussion

Failure of remyelination is a fundamental problem in MS and contributes to the long-term decline of neuroaxonal function, the most prominent and therapeutically least targetable feature of the disease. Although recent work proposes a role for myelin in predisposing axons to damage in the context of autoimmune inflammation, the ability of oligodendrocytes and myelin to provide physical protection and metabolic support is undisputed.[42,43] Which cells are at the forefront of myelin regeneration is a matter of ongoing debate.[44,45] Previous studies in human post-mortem tissue and experimental models suggested that remyelination after a demyelinating insult is more efficient in the cerebral cortex compared to the white matter. This raises the question as to the underlying cellular and molecular mechanisms. In the present work, we demonstrated that cortical BCAS1+ oligodendrocytes, in part localized in a perineuronal satellite position, engage in remyelination in demyelinating models and human biopsy and autopsy tissue from patients with MS. This supports the notion that a defined and pre-differentiated pre-myelinating oligodendrocyte population is available in the cortex to enable rapid and efficient remyelination.

Demyelination of the cortical grey matter is an important characteristic of MS, and the proportion of demyelinated grey matter outweighs that of demyelinated white matter in many patients.[46,47] However, cortical remyelination efficacy is prominent when areas of remyelination in cortical and white matter lesions are compared in the same patients.[15-17] Studies in human tissue and experimental models suggest that numerical and functional differences in oligodendrocyte lineage cells, as well as e.g. the phenotype of reactive astrogliosis, including a lower expression of CD44, versican and hyaluronan in the cortex, may play a role.[15] Also, oligodendrocyte precursor cells in cortex and white matter were shown to differ in their propensity for terminal differentiation, and cortical oligodendrocytes reach their final number much later in life than in the white matter, as inferred from ^{14}C incorporation.[48] In previous work, we demonstrated that the density of BCAS1+ oligodendrocytes in the cortex remains high throughout life—in stark contrast to the white matter—supporting a physiological role in neuronal function, e.g. as effectors of adaptive cortical myelination and circuit modulation.[49,50] Here, we have shown that remyelination in human cortical MS lesions and animal models of toxic and inflammatory cortical demyelination is characterized by a morphological change of BCAS1+ oligodendrocytes from a resting towards an activated phenotype. Our results indicated that resting resp. pre-differentiated cortical BCAS1+ oligodendrocytes develop into internode-forming, MAG-expressing cells via a branched, star-like pre-myelinating phenotype and down-regulate BCAS1 expression upon differentiation to myelin-maintaining oligodendrocytes. This suggested that cortical BCAS1+ oligodendrocytes contribute to remyelination after a demyelinating insult to the cortex and may constitute a 'quiescent' population of pre-differentiated oligodendrocytes that serves as a reservoir for swift and efficient myelin regeneration.

Interestingly, our study revealed that around 40% of BCAS1+ oligodendrocytes in mouse and human were localized in a perineuronal satellite position. Satellite oligodendrocytes are largely considered non-myelinating and may fulfil specific functions in neuronal support.[51-54] However, data from the cuprizone mouse model and *in vivo* imaging suggests that these cells can generate compact myelin around pyramidal neurons of mouse layer V.[54,55] Importantly, satellite oligodendrocytes sense neuronal electric and metabolic activity, thus putting them into an ideal position to react rapidly to changing metabolic and circuitry demands. Our study also demonstrated that the positionally defined subpopulation of BCAS1+ perineuronal satellite oligodendrocytes can assume a myelinating morphology and express MAG in both the mouse cuprizone model and MS lesions. This indicates that these highly specialized, physiologically mostly non-myelinating oligodendrocytes can indeed be recruited for remyelination.

In our experimental and human tissue studies, we have found that the density of myelinating BCAS1+ oligodendrocytes correlates

Figure 4 Continued
with Tukey's multiple comparison test; $^{**}P < 0.01$, $^{***}P < 0.001$). (**E**) Co-labelling of MBP with tubulin polymerization promoting protein (TPPP/p25) and Olig2. Arrowheads indicate TPPP/p25+ and Olig2+ cells in the NC and NAC. (**F**) Quantification of TPPP/p25+ cells (NC, $n = 16$; NAC, $n = 13$; DM, $n = 8$; RM, $n = 4$; one-way ANOVA with Tukey's multiple comparison test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) and (**G**) Olig2+ cells in the cortex of MS patients and age-matched controls (NC, $n = 12$; NAC, $n = 9$; DM, $n = 9$; RM, $n = 4$; Kruskal–Wallis test with Dunn's multiple comparison test; $^{*}P < 0.05$); mean ± standard error of the mean. Scale bars in **A** = 100 µm and **E** = 50 µm.

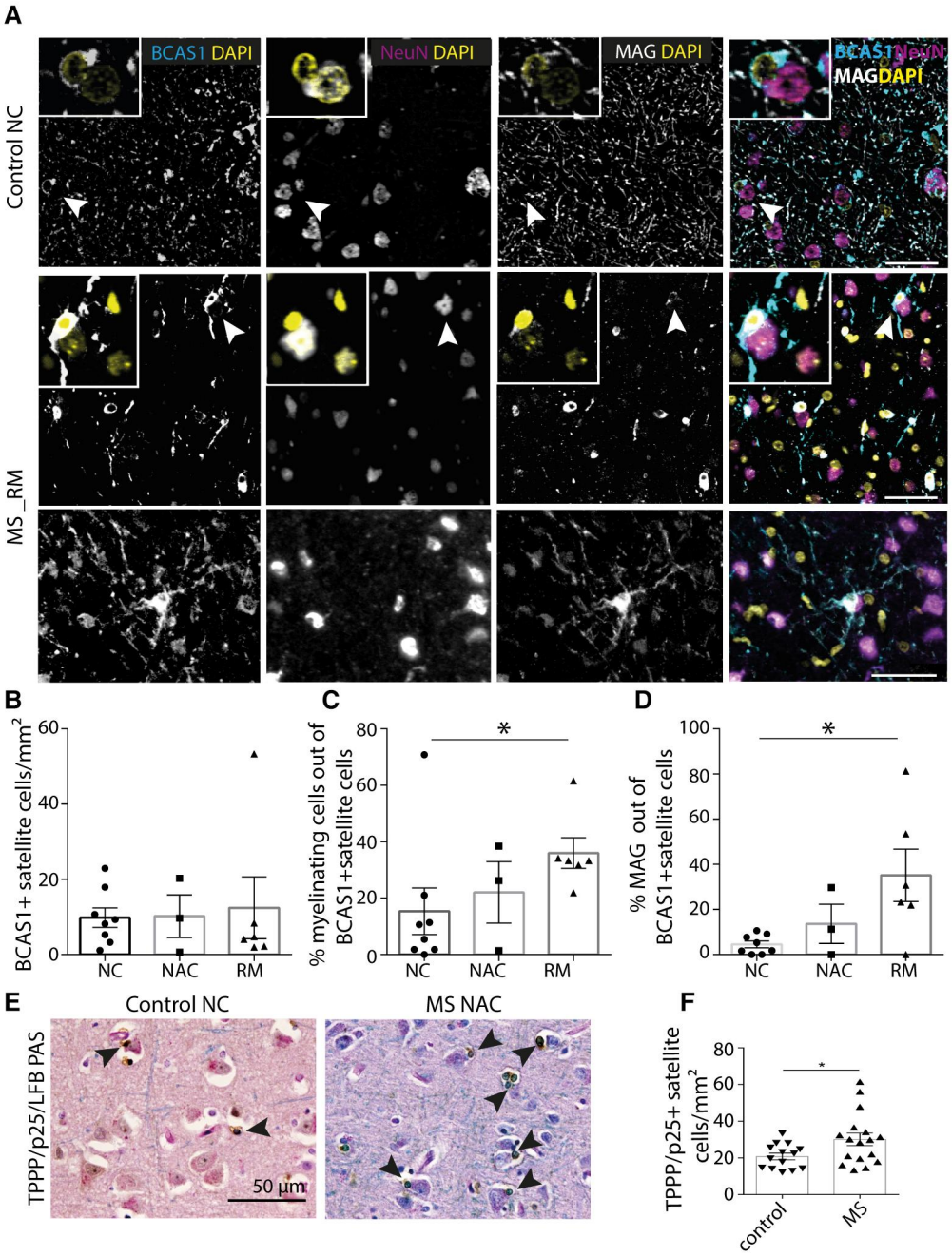

Figure 5 BCAS1+ satellite oligodendrocytes myelinate early cortical lesions of multiple sclerosis. (**A**) Triple immunofluorescence labelling of BCAS1 (cyan) with NeuN (neuronal nuclear antigen, magenta) and the myelin-associated glycoprotein (MAG, white) of control cortex (control, NC) and biopsies from patients with multiple sclerosis (MS) that show active, ongoing remyelination (RM). *Insets*: BCAS1+ perineuronal satellite oligodendrocytes, co-expressing MAG in cortical remyelination, as indicated by the arrowhead. (**B**) Density of BCAS1+ satellite oligodendrocytes in control biopsies and patients with MS [NC, $n = 8$; normal-appearing cortex (NAC), $n = 3$; RM, $n = 6$]. (**C**) Density of BCAS1+ satellite oligodendrocytes with a process-rich myelinating morphology as percentage of the total number of BCAS1+ satellite oligodendrocytes in control biopsies and patients with MS (NC, $n = 8$; NAC, $n = 3$; RM, $n = 6$; Kruskal–Wallis test with Dunn's multiple comparison test; $^*P < 0.05$). (**D**) Percentage of BCAS1+ satellite oligodendrocytes that expresses MAG in control biopsies and patients with MS (NC, $n = 8$; NAC, $n = 3$; RM, $n = 6$; Kruskal–Wallis test with Dunn's multiple comparison test; $^*P < 0.05$). (**E**) Luxol Fast Blue periodic acid-Schiff staining and TPPP/p25 immunohistochemistry (IHC) of autopsies from controls and patients with progressive MS. Arrowheads indicate TPPP/p25+ satellite oligodendrocytes. (**F**) Density of TPPP/p25+ satellite oligodendrocytes in controls and the NAC of patients with MS (controls, $n = 14$; MS, $n = 18$; Mann–Whitney test; $^*P < 0.05$). Error bars indicate mean ± standard error of the mean. Scale bars = 50 µm.

with the presence of myeloid cells. For cortical grey matter, this now indicates that oligodendrocyte recruitment and remyelination is an early phenomenon in MS lesion formation that occurs in the immediate post-phagocytic phase when foam cells are still abundant in the demyelinated lesions.[56-60] This aligns with the notion that the debris-clearing function of myeloid cells and mediators released from innate immune cells stimulate remyelination.[61-63] Recent data have indicated that the differentiation of local oligodendrocyte progenitor cells into actively myelinating oligodendrocytes is stimulated by innate immune cells.[64-67] This may also be true for the quiescent

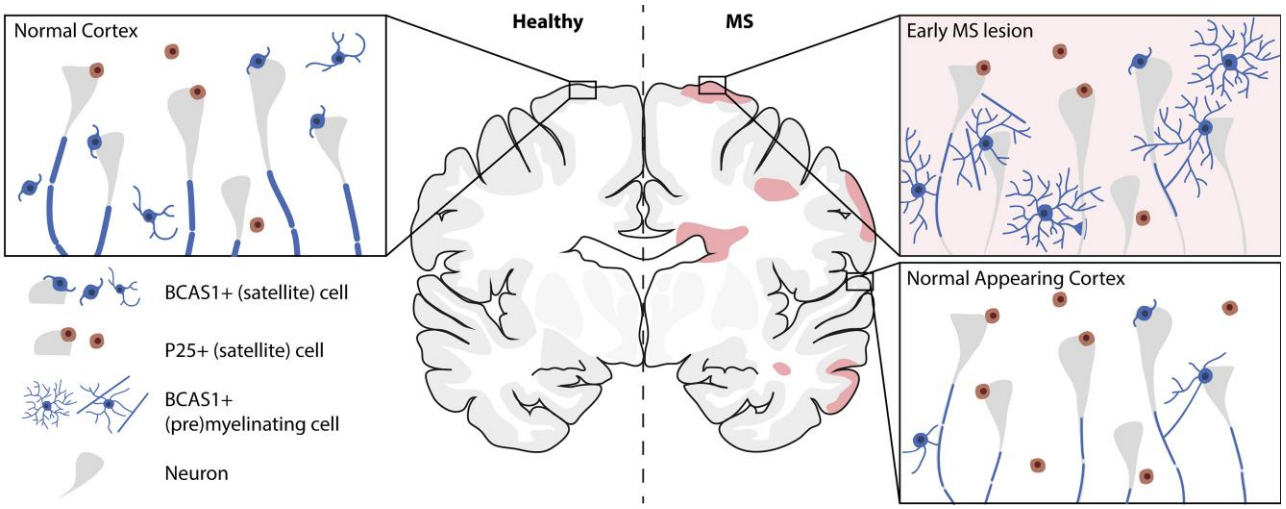

Figure 6 BCAS1+ oligodendrocytes, including perineuronal BCAS1+ satellite oligodendrocytes, participate in remyelination after cortical demyelination in multiple sclerosis. Schematic illustration of BCAS1+ and TPPP/p25+ oligodendrocytes in the cortex of a healthy brain compared to early cortical demyelination and the normal-appearing cortex in multiple sclerosis (MS). In the normal adult cortex, we mainly find BCAS1+ oligodendrocytes with few or no processes, including perineuronal non-myelinating satellite cells. These cells are activated early after demyelination in MS lesions and differentiate to BCAS1+ cells with a pre- and myelinating morphology to generate new myelin, while the total number of BCAS1+ oligodendrocytes remains unchanged. Finally, these cells differentiate to mature, TPPP/p25+ myelin-maintaining oligodendrocytes. Increased numbers of TPPP/p25+ oligodendrocytes and reduced numbers of BCAS1+ oligodendrocytes may indicate that areas of normal appearing cortex have been subjected to differentiation stimuli, e.g. inflammation or demyelination.

population of pre-differentiated BCAS1+ oligodendrocytes. Importantly, our data thus also suggest that the presence of actively myelinating BCAS1+ oligodendrocytes can be used as an indicator to identify regions of ongoing MS-related myelin damage in the cortex.

Although remyelination of cortical MS lesions is more efficient than that of white matter lesions, autopsy studies of patients with long-lasting disease show extensive cortical demyelination with severe reduction in oligodendrocyte lineage cells.[41,46,68] Our data on the normal-appearing cortex in chronic MS, with morphologically inconspicuous myelination as assessed by MBP immunohistochemistry, revealed an important reduction of pre-differentiated BCAS1+ oligodendrocytes compared to age-matched controls. However, the density of Olig2+ cells, reflecting the entire oligodendroglial lineage, remained unperturbed. Interestingly, the reduction in BCAS1+ oligodendrocytes was accompanied by an increase in TPPP/p25+ mature, myelin-maintaining oligodendrocytes, suggesting a shift towards more mature subsets of oligodendrocytes. This may reflect previous stimulation of oligodendroglial differentiation in the normal-appearing cortex in the context of disease, with differentiation of BCAS1+ oligodendrocytes, including satellite cells, to myelin-maintaining oligodendrocytes (Fig. 6). Interestingly, increased densities of TPPP/p25 myelin-maintaining oligodendrocytes have previously been demonstrated in the normal-appearing white matter in autopsy tissue of patients with chronic MS.[69] Of note, in previous work we found evidence of higher g-ratios of cortical myelinated fibres in MS, suggesting that part of the microscopically normal-appearing cortex has experienced a loss of oligodendrocytes, with ensuing remyelination.[17] Taken together, these data suggest that ongoing MS-related cortical tissue damage, in particular related to demyelination and the exposure to inflammatory mediators, could explain the reduction in regeneration capacity after long-lasting disease. In the cortex, such damage could cause premature terminal differentiation of early oligodendrocyte developmental stages serving immediate tissue repair. Thus, while we observe a higher propensity to remyelinate in early disease, failure of repair ensues if further and long-lasting damage is imposed on the cortex, eventually resulting in persisting demyelination, as frequently observed in autopsy tissue of later stage disease.

An interesting question in this context is whether expression of BCAS1 is a feature of all pre- and actively myelinating oligodendrocytes, whether derived from parenchymal oligodendrocyte precursor cells or neurogenic zones such as the subventricular zone.[26,70] Further experimental work, including lineage tracing experiments and an assessment of internode length and g-ratio will be required to address this issue. Previous experimental and human work on demyelinated cortex suggests that g-ratios are indeed higher and internodes shorter than in NC, however, without directly associating newly formed internodes to the expression of BCAS1.[10,17]

In summary, in combined work on MS patient tissues and experimental models of demyelination, we provide evidence that the cerebral cortex harbours a population of BCAS1+ pre-differentiated oligodendrocytes ready to myelinate in case of a demyelinating insult, such as occurs in MS. This ‘quiescent’ cell population that can be mobilized without cell division might for a large part explain the higher remyelination efficacy in the cortex of patients with MS. However, we also demonstrate here that pre-differentiated BCAS1+ oligodendrocytes are decreased in later stage MS, potentially impairing the swift and efficient cortical remyelination response observed in early disease. Further studies on the dynamics of cortical oligodendroglia populations in MS, including lineage tracing experiments in models of demyelination, will reveal the complex interactions between glial cells and neurons in demyelination and their likely contribution to neuronal dysfunction in progressive MS.

Data availability

All original data are available from the authors upon reasonable request.

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Competing interests

The authors declare that they have no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Irvine KA, Blakemore WF. Remyelination protects axons from demyelination-associated axon degeneration. *Brain*. 2008;131:1464-1477.
2. Duncan ID, Brower A, Kondo Y, Curlee JF, Schultz RD. Extensive remyelination of the CNS leads to functional recovery. *Proc Natl Acad Sci U S A*. 2009;106:6832-6836.
3. Fünfschilling U, Supplie LM, Mahad D, et al. Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature*. 2012;485:517-521.
4. Lee Y, Morrison BM, Li Y, et al. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature*. 2012;487:443-448.
5. Lassmann H, Brück W, Lucchinetti C, Rodriguez M. Remyelination in multiple sclerosis. *Mult Scler*. 1997;3:133-136.
6. Prineas JW, Connell F. Remyelination in multiple sclerosis. *Ann Neurol*. 1979;5:22-31.
7. Graves JS, Krysko KM, Hua L, et al. Ageing and multiple sclerosis. *Lancet Neurol*. 2023;22:66-77.
8. Orthmann-Murphy J, Call CL, Molina-Castro GC, et al. Remyelination alters the pattern of myelin in the cerebral cortex. *Elife*. 2020;9:e56621.
9. Merkler D. A new focal EAE model of cortical demyelination: Multiple sclerosis-like lesions with rapid resolution of inflammation and extensive remyelination. *Brain*. 2006;129:1972-1983.
10. Snaidero N, Schifferer M, Mezydlo A, Zalc B, Kerschensteiner M, Misgeld T. Myelin replacement triggered by single-cell demyelination in mouse cortex. *Nat Commun*. 2020;11:141.
11. Patrikios P, Stadelmann C, Kutzelnigg A, et al. Remyelination is extensive in a subset of multiple sclerosis patients. *Brain*. 2006;129(Pt 12):3165-3172.
12. Chang A, Tourtellotte WW, Rudick R, Trapp BD. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. *N Engl J Med*. 2002;346:165-173.
13. Sim FJ, Zhao C, Penderis J, Franklin RJM. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J Neurosci*. 2002;22:2451-2459.
14. Kuhlmann T, Miron V, Cuo Q, Wegner C, Antel J, Bruck W. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. *Brain*. 2008;131:1749-1758.
15. Chang A, Staugaitis SM, Dutta R, et al. Cortical remyelination: A new target for repair therapies in multiple sclerosis. *Ann Neurol*. 2012;72:918-926.
16. Strijbis EM, Kooi E-J, van der Valk P, Geurts JJ. Cortical remyelination is heterogeneous in multiple sclerosis. *J Neuropathol Exp Neurol*. 2017;76:390-401.
17. Albert M, Antel J, Brück W, Stadelmann C. Extensive cortical remyelination in patients with chronic multiple sclerosis. *Brain Pathol*. 2007;17:129-138.
18. Lazzarotto A, Hamzaoui M, Tonietto M, et al. Time is myelin: Early cortical myelin repair prevents atrophy and clinical progression in multiple sclerosis. *Brain*. 2024;147:1331-1343.
19. Duncan ID, Radcliff AB, Heidari M, Kidd G, August BK, Wierenga LA. The adult oligodendrocyte can participate in remyelination. *Proc Natl Acad Sci U S A*. 2018;115:E11807-E11816.
20. Jäkel S, Agirre E, Mendanha Falcão A, et al. Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature*. 2019;566:543-547.
21. Lubetzki C, Gansmüller A, Lachapelle F, Lombrail P, Gumpel M. Myelination by oligodendrocytes isolated from 4–6-week-old rat central nervous system and transplanted into newborn shiverer brain. *J Neurol Sci*. 1988;88:161-175.
22. Yeung MS, Zdunek S, Bergmann O, et al. Dynamics of oligodendrocyte generation and myelination in the human brain. *Cell*. 2014;159:766-774.
23. Gensert JM, Goldman JE. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. *Neuron*. 1997;19:197-203.
24. Carroll WM, Jennings AR. Early recruitment of oligodendrocyte precursors in CNS demyelination. *Brain*. 1994;117:563-578.
25. Windrem MS, Nunes MC, Rashbaum WEEK, et al. Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nat Med*. 2004;10:93-97.
26. Xing YL, Roth PT, Stratton JAS, et al. Adult neural precursor cells from the subventricular zone contribute significantly to oligodendrocyte regeneration and remyelination. *J Neurosci*. 2014;34:14128-14146.
27. Neely SA, Williamson JM, Klingseisen A, et al. New oligodendrocytes exhibit more abundant and accurate myelin regeneration than those that survive demyelination. *Nat Neurosci*. 2022;25:415-420.
28. Mezydlo A, Treiber N, Ullrich Gavilanes EM, et al. Remyelination by surviving oligodendrocytes is inefficient in the inflamed mammalian cortex. *Neuron*. 2023;111:1748-1759.e8.
29. Fard MK, van der Meer F, Sánchez P, et al. BCAS1 expression defines a population of early myelinating oligodendrocytes in multiple sclerosis lesions. *Sci Transl Med*. 2017;9:eaam7816.
30. Ishimoto T, Ninomiya K, Inoue R, Koike M, Uchiyama Y, Mori H. Mice lacking BCAS1, a novel myelin-associated protein, display hypomyelination, schizophrenia-like abnormal behaviors, and

upregulation of inflammatory genes in the brain. *Glia*. 2017;65:727-739.
31. Kaji S, Maki T, Ueda J, et al. BCAS1-positive immature oligodendrocytes are affected by the α-synuclein-induced pathology of multiple system atrophy. *Acta Neuropathol Commun*. 2020;8:120.
32. Brück W, Porada P, Poser S, et al. Monocyte/macrophage differentiation in early multiple sclerosis lesions. *Ann Neurol*. 1995;38:788-796.
33. Litzenburger T, Fässler R, Bauer J, et al. B lymphocytes producing demyelinating autoantibodies: Development and function in gene-targeted transgenic mice. *J Exp Med*. 1998;188:169-180.
34. Kerschensteiner M, Bareyre FM, Buddeberg BS, et al. Remodeling of axonal connections contributes to recovery in an animal model of multiple sclerosis. *J Exp Med*. 2004;200:1027-1038.
35. Nessler S, Boretius S, Stadelmann C, et al. Early MRI changes in a mouse model of multiple sclerosis are predictive of severe inflammatory tissue damage. *Brain*. 2007;130:2186-2198.
36. Lagumersindez-Denis N, Wrzos C, Mack M, et al. Differential contribution of immune effector mechanisms to cortical demyelination in multiple sclerosis. *Acta Neuropathol*. 2017;134:15-34.
37. Bergner CG, Genc N, Hametner S, et al. Concurrent axon and myelin destruction differentiates X-linked adrenoleukodystrophy from multiple sclerosis. *Glia*. 2021;69:2362-2377.
38. Jiang G, Ayaki T, Maki T, et al. Evaluation of BCAS1-positive immature oligodendrocytes after cerebral ischemic stroke and SVD. *Neurosci Lett*. 2023;812:137405.
39. del Rio Hortega P. La neuroglía normal. Conceptos de neuroglíona y angiogliona. *Arch Histol Norm Patol*. 1942;1:5-71.
40. Kuhlmann T, Ludwin S, Prat A, Antel J, Brück W, Lassmann H. An updated histological classification system for multiple sclerosis lesions. *Acta Neuropathol*. 2017;133:13-24.
41. Rodriguez EG, Wegner C, Kreutzfeldt M, et al. Oligodendroglia in cortical multiple sclerosis lesions decrease with disease progression, but regenerate after repeated experimental demyelination. *Acta Neuropathol*. 2014;128:231-246.
42. Schäffner E, Bosch-Queralt M, Edgar JM, et al. Myelin insulation as a risk factor for axonal degeneration in autoimmune demyelinating disease. *Nat Neurosci*. 2023;26:1218-1228.
43. Franklin RJM, Bodini B, Goldman SA. Remyelination in the central nervous system. *Cold Spring Harb Perspect Biol*. 2024;16:a041371.
44. Franklin RJ, Frisén J, Lyons DA. Revisiting remyelination: Towards a consensus on the regeneration of CNS myelin. *Semin Cell Dev Biol*. 2021;116(Pt 3):3-9.
45. Lubetzki C, Zalc B, Williams A, Stadelmann C, Stankoff B. Remyelination in multiple sclerosis: From basic science to clinical translation. *Lancet Neurol*. 2020;19:678-688.
46. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain*. 2005;128(Pt 11):2705-2712.
47. Trapp BD, Vignos M, Dudman J, et al. Cortical neuronal densities and cerebral white matter demyelination in multiple sclerosis: A retrospective study. *Lancet Neurol*. 2018;17:870-884.
48. Yeung MSY, Djelloul M, Steiner E, et al. Dynamics of oligodendrocyte generation in multiple sclerosis. *Nature*. 2019;566:538-542.
49. Gibson EM, Purger D, Mount CW, et al. Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. *Science*. 2014;344:1252304.
50. McKenzie IA, Ohayon D, Li H, et al. Motor skill learning requires active central myelination. *Science*. 2014;346:318-322.
51. Takasaki C, Yamasaki M, Uchigashima M, Konno K, Yanagawa Y, Watanabe M. Cytochemical and cytological properties of perineuronal oligodendrocytes in the mouse cortex. *Eur J Neurosci*. 2010;32:1326-1336.
52. Szuchet S, Nielsen JA, Lovas G, et al. The genetic signature of perineuronal oligodendrocytes reveals their unique phenotype. *Eur J Neurosci*. 2011;34:1906-1922.
53. Kruger L, Maxwell DS. Electron microscopy of oligodendrocytes in normal rat cerebrum. *Am J Anat*. 1966;118:411-435.
54. Battefeld A, Klooster J, Kole MHP. Myelinating satellite oligodendrocytes are integrated in a glial syncytium constraining neuronal high-frequency activity. *Nat Commun*. 2016;7:232.
55. Ludwin SK. The perineuronal satellite oligodendrocyte. *Acta Neuropathol*. 1979;47:49-53.
56. Raine CS, Wu E. Multiple sclerosis: Remyelination in acute lesions. *J Neuropathol Exp Neurol*. 1993;52:199-204.
57. Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES. Multiple sclerosis: Remyelination of nascent lesions. *Ann Neurol*. 1993;33:137-151.
58. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. *Brain*. 1999;122:2279-2295.
59. Brück W, Schmied M, Suchanek G, et al. Oligodendrocytes in the early course of multiple sclerosis. *Ann Neurol*. 1994;35:65-73.
60. Prineas JW, Lee S. Microglia subtypes in acute, subacute, and chronic multiple sclerosis. *J Neuropathol Exp Neurol*. 2023;82:674-694.
61. Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci*. 2001;4:1116-1122.
62. Foote AK. Inflammation stimulates remyelination in areas of chronic demyelination. *Brain*. 2005;128:528-539.
63. Syed YA, Zhao C, Mahad D, et al. Antibody-mediated neutralization of myelin-associated EphrinB3 accelerates CNS remyelination. *Acta Neuropathol*. 2016;131:281-298.
64. Miron VE, Boyd A, Zhao J-W, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci*. 2013;16:1211-1218.
65. Madsen PM, Motti D, Karmally S, et al. Oligodendroglial TNFR2 mediates membrane TNF-dependent repair in experimental autoimmune encephalomyelitis by promoting oligodendrocyte differentiation and remyelination. *J Neurosci*. 2016;36:5128-5143.
66. Cunha MI, Su M, Cantuti-Castelvetri L, et al. Pro-inflammatory activation following demyelination is required for myelin clearance and oligodendrogenesis. *J Exp Med*. 2020;217:e20191390.
67. Franklin RJM, Simons M. CNS remyelination and inflammation: From basic mechanisms to therapeutic opportunities. *Neuron*. 2022;110:3549-3565.
68. Bø L. The histopathology of grey matter demyelination in multiple sclerosis. *Acta Neurol Scand Suppl*. 2009;189:51-57.
69. Höftberger R, Fink S, Aboul-Enein F, et al. Tubulin polymerization promoting protein (TPPP/p25) as a marker for oligodendroglial changes in multiple sclerosis. *Glia*. 2010;58:1847-1857.
70. Remaud S, Ortiz FC, Perret-Jeanneret M, et al. Transient hypothyroidism favors oligodendrocyte generation providing functional remyelination in the adult mouse brain. *Elife*. 2017;6:e29996.