Thinking outside the box: non-canonical targets in multiple sclerosis

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Abstract | Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system that causes demyelination, axonal degeneration and astrogliosis, resulting in progressive neurological disability. Fuelled by an evolving understanding of MS immunopathogenesis, the range of available immunotherapies for clinical use has expanded over the past two decades. However, MS remains an incurable disease and even targeted immunotherapies often fail to control insidious disease progression, indicating the need for new and exceptional therapeutic options beyond the established immunological landscape. In this Review, we highlight such non-canonical targets in preclinical MS research with a focus on five highly promising areas: oligodendrocytes; the blood–brain barrier; metabolites and cellular metabolism; the coagulation system; and tolerance induction. Recent findings in these areas may guide the field towards novel targets for future therapeutic approaches in MS.

Multiple sclerosis (MS) is the most frequently occurring neuroinflammatory disease and the commonest cause of permanent disability in younger adults. The aetiology of the disease remains elusive but a large body of evidence suggests that it is immune-mediated in nature. Globally, some 2.8 million people are affected and its incidence and prevalence have been on the rise worldwide over the past few decades. In the majority of patients, the disease takes a relapsing course with intermittent periods of neurological dysfunction that may initially completely resolve; however, as the disease advances, recovery is incomplete and disability accumulates. In addition, progression may occur independently of relapse activity. In two-thirds of cases, the transition from this relapsing course to secondary progressive MS usually occurs after 10–15 years and, for some time, superimposed relapses may occur, reflecting ongoing inflammatory activity. 10%–15% of patients follow a primary progressive course, characterized by the continuous worsening of neurological disability from the first manifestation of disease. These descriptions of the course of the disease have recently been modified to classify MS into either relapsing forms, with disability occurring both in relation to and independently of relapses, or progressive forms that are active or inactive, classified according to clinical or magnetic resonance imaging (MRI) findings (BOX 1).

Typical MS manifestations include visual, sensory, motor and sphincter disturbances, as well as incoordination, gait disorder and cognitive impairment. The disease spans decades and life expectancy is shortened. MS therefore places a heavy burden on patients, their families and caregivers, healthcare systems and society at large.

Cardinal pathological features are multifocal inflammation, primary demyelination, oligodendroglial death, neuroaxonal degeneration, and astrocytic scarring in the brain and spinal cord. Both white and grey matter are affected. Axonal damage detectable early in the course of the disease foretells the development of permanent disability; tissue destruction gives rise to global and regional brain and spinal cord atrophy. During the early stages of the disease, the ongoing damage may go unnoticed as compensatory functional mechanisms are recruited, involving supplementary neuronal circuitry and the capacity for remyelination of damaged dysfunctional axons by oligodendrocyte precursors.

Early disease pathology is led by adaptive immunity outside the central nervous system (CNS). As the disease evolves, adaptive immunity loses importance and CNS-specific innate immunity, orchestrated by microglia and astroglia, takes precedence. Axonal damage and neurodegeneration may be caused by collateral damage in the wake of a vigorous inflammatory response, with demyelination rendering the denuded axon susceptible to noxious mediators, lack of neurotrophic factors and retrograde degeneration. Molecular effector pathways involve oxidative stress, calcium...

[End of extract]
Neuronal damage or death that is caused by excessive release of neurotransmitters such as glutamate or aspartate.

Experimental autoimmune encephalomyelitis (EAE). An inflammatory, autoimmune demyelinating disease of the central nervous system in rodents that has high pathological and clinical similarities to human multiple sclerosis and is the most used experimental model for the disease.

Although the disease remains incurable, the past three decades have witnessed the successful development of disease-modifying therapies (DMTs), predominantly for the relapsing forms [11-15] (fig. 1). DMTs predominantly curtail the migration of lymphocytes into the CNS, or deplete specific types of immune cell. Recently, siponimod (which modulates cell migration) was the first agent to provide a moderate benefit in secondary progressive MS, and ocrelizumab (which depletes B cells) was the first drug to be moderately effective in a subgroup of patients with primary progressive MS (PPMS) [1].

Nevertheless, there remain major unmet needs. First, for the majority of patients with relapsing disease, DMTs fail to generate sustained control of disease activity. Second, for the majority of patients with progressive disease, no sufficiently effective treatment is available. Although the conversion rate to secondary progression is reduced with DMTs [16,17], roughly 10–15% of patients develop secondary progressive disease after 5 years [18]; the long-term risk for conversion to SPMS (15 years) with DMTs is about 34% [19]. This emphasizes the urgent need for drugs fostering remyelination and repair, and thereby promoting improvement in disability.

All approved DMTs are fundamentally anti-inflammatory and/or immunomodulatory. An enhanced understanding of the immunological and neurobiological underpinnings of MS may open new avenues for therapeutic research, utilizing non-canonical pathways to improve outcomes. In this Review, we select research areas that have demonstrated substantial progress in recent years, hold great promise to identify new molecular targets in MS and may allow the design of more specific and effective therapeutic strategies in the future. We focus on five fields of research (presented in descending order of greatest future therapeutic potential): oligodendrocytes, the blood–brain barrier (BBB), metabolites and cellular metabolism, the coagulation system and tolerance induction.

### Pathophysiology and therapeutics

Persistent inflammation is key to MS pathophysiology, and a better understanding of the inflammatory cascade — both peripherally and in the CNS — is critical to establishing novel therapeutic targets.

MS is considered to be an immune-mediated disease caused by the activation of T and B lymphocytes that act against CNS antigens. Breakdown of tolerance to autoantigens allows previously dormant autoreactive T and B cells to become activated. Similar to most autoimmune diseases, the triggering event is not known in MS, but several studies indicate that genetic background plays a part by tuning the adaptive immune response, which alters immunological activation thresholds as well as the efficacy of immunoregulatory pathways. A robust genetic link has been repeatedly found between MS and certain human leukocyte antigen (HLA)-encoded class II major histocompatibility complex (MHC) molecules [20]. In particular, in recent ex vivo studies using samples from patients with MS, HLA-DR15 haplotypes were instrumental in orchestrating an autoimmune response against the brain and spinal cord, mediated by T cells and fuelled by B cells [21].

According to these insights, which have been substantiated in variants of experimental autoimmune encephalomyelitis (EAE) [22,23], activated myelin-reactive T lymphocytes and other immune cells infiltrate brain tissue by crossing the BBB. Subsequently, autoreactive T cells are locally reactivated by classical or tissue-resident antigen-presenting cells — including perivascular macrophages, dendritic cells and microglia — and by a surge of pro-inflammatory mediators, including cytokines and chemokines, that are released by immune and glial cells. This results in further immune cell recruitment and amplification of the inflammatory cascade in the CNS. Furthermore, macrophage and/or microglial activation leads to myelin destruction both directly and by antibody- or autoantibody-mediated phagocytosis of the myelin sheath, resulting in demyelination, axonal degeneration, neuronal dysfunction and consequent neurodegeneration [24]. Thus, ongoing

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**Box 1 | Different forms of MS**

The disease course of multiple sclerosis (MS) takes three main forms. The common is relapsing-remitting MS (RRMS, approximately 85%), which is characterized by fully or partially reversible phases of neurological disability (see the figure, part a). About two-thirds of patients with RRMS develop progressive disease, that is, secondary progressive MS (SPMS) with a continuously progressing disability (see the figure, part b). Both RRMS and SPMS can be further characterized as either active (showing new relapses or evidence of new MRI activity) or non-active (where no evidence of disease activity occurs), as well as worsening (increased disability following a relapse) or non-worsening. In the third form, primary progressive MS (PPMS), disease activity continuously worsens from the onset, and this course can be further differentiated into active (evidence of new MRI activity) or non-active, as well as with progression (disability accumulation over time) or without progression (see the figure, part c). A prodromal stage has been described and, on occasion, characteristic imaging features of MS can be incidentally identified before the disease becomes obvious clinically. This is referred to as RIS, radiologically isolated syndrome [25].

- **a** RRMS
  - Disability
  - Time
  - RIS/CIS
  - Relapse
  - Active without worsening
  - Worsening
  - Stable without activity
  - RRMS
  - Active with progression
  - Active without progression
  - Not active with progression
  - Not active without progression
  - New MRI activity

- **b** SPMS
  - Disability
  - Time

- **c** PPMS
  - Disability
  - Time

CIS, clinically isolated syndrome. Figure adapted with permission from the National MS Society, based on data from REF. [26].
activation of autoreactive T cells critically contributes to repeated and deleterious waves of inflammation targeting the brain and spinal cord.

Conceptually, the HLA-DR15 haplotype, which predisposes individuals to develop MS, physically interacts with both endogenous myelin autoantigens and MS-associated foreign antigens. The latter encompasses antigens derived from gut-derived microbes, such as Akkermansia muciniphila and Acinetobacter calcoaceticus, as well as the Epstein–Barr virus. The Epstein–Barr virus is of particular interest as it latently infects and immortalizes B lymphocytes, driving their persistence in an activated state and possibly contributing to MS pathogenesis by generating a pro-inflammatory milieu and forming ectopic lymphoid follicles in the CNS.

These insights and the unexpectedly high efficacy of B cell-depleting immunotherapies have flagged B cells as key players in MS pathophysiology. B cells can present antigens to T cells and thereby drive their clonal proliferation and the production of pro-inflammatory cytokines. Long-lived tissue-resident B cells are located within the meninges, and in vivo neuroinflammation augments their antigen-presenting capacity. We note that meningeal T cell infiltration has been seen before the appearance of clinical signs and even prior to dissemination into the CNS parenchyma, indicating that this initial step is a potential T cell checkpoint in early disease pathology. In addition, subpial aggregates of B cells and CD8+ T cells have been implicated as drivers of compartmentalized inflammation during the progressive stages of the disease.

Currently approved DMTs primarily target the aberrant immune response in the peripheral immune system to effectively reduce episodes of inflammatory demyelination. Having fewer inflammatory episodes provides indirect — or secondary — neuroprotection by restricting subsequent neurodegeneration and thus preventing neurological disability. DMTs may be categorized according to the underlying mode of action: pleiotropic effects, reduced immune cell proliferation, targeted depletion of immune cells or reduced immune cell migration.

A number of established MS immunotherapies have pleiotropic effects. This is best demonstrated by mild but well established immunomodulatory agents such as interferon-β (IFNβ), glatiramer acetate and dimethyl-fumarate. IFNβ is an endogenous cytokine. Its potent antiviral responses include down-regulation of MHC class II expression, interference with T cell homeostasis...
and inhibition of adhesion molecules, thus stabilizing the BBB\textsuperscript{39}. Glatiramer acetate is a complex mixture of random peptides that mimic major myelin proteins, and administration produces a mild but persistent attenuation of the pro-inflammatory phenotype, mainly by affecting the autoaggressive lymphocyte population that targets the CNS myelin sheath\textsuperscript{39}. For dimethylfumarate, multiple molecular targets have been suggested, including nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor involved in both lymphogenesis\textsuperscript{41} and the oxidative stress response\textsuperscript{42}. These pleiotropic therapies are effective only for relapsing forms of MS\textsuperscript{43}.

Nonspecific immunosuppressants derived from classical chemotherapeutic agents, such as azathioprine, mitoxantrone and cyclophosphamide, which are not routinely used for MS, indiscriminately reduce the proliferation of all rapidly dividing cells, including immune cells. As a consequence, both physiological immune functions (such as protection from pathogens and cancer prevention) and pathological autoimmune activity are reduced; the therapeutic benefits for MS are achieved at the expense of long-term side effects including the increased risk of therapy-related secondary cancers\textsuperscript{44}. Furthermore, at least for the better tolerated nonspecific immunosuppressants such as azathioprine, the therapeutic effects are rather modest\textsuperscript{45}. Therefore, those therapies are generally associated with an unfavourable risk-to-benefit ratio. Although it is often considered to be a broad immunosuppressant, teriflunomide, which inhibits dihydroorotate dehydrogenase, probably has a more lymphocyte-specific mode of action, as comprehensive immunosuppressive effects have so far not been observed\textsuperscript{46}.

Building on the rationale of using nonspecific immunosuppressants, depletion of select immune cell populations has been systematically explored and established in MS. A single cycle of alemtuzumab, which targets CD52, a pan-lymphocyte cell-surface molecule, removes all lymphocytes; B lymphocytes recover quickly (sometimes exceeding previous levels), whereas T lymphocytes are not detectable for up to 18 months\textsuperscript{47}. A complementary approach is the use of anti-CD20 antibodies such as rituximab, ocrelizumab and ofatumumab, which deplete most cells of the B lymphocyte lineage and a small population of T cells\textsuperscript{48}. A study in a PPMS cohort demonstrated moderate beneficial effects of rituximab in the inflammatory stages of PPMS\textsuperscript{49}, prompting a randomized phase III placebo-controlled trial of the humanized anti-CD20 monoclonal antibody ocrelizumab. The results led to the first approval of a drug for PPMS\textsuperscript{50}. In a third approach, by inhibiting lymphocyte-specific signalling cascades, pulsed oral cladribine is able to remove both B and T cells to a similar degree\textsuperscript{48}. All three depletion strategies may be classified as selective immunosuppression and have superior efficacy to the milder immunotherapies described above. For alemtuzumab and cladribine, reconstitution of a normalized immune system following the initial depletion of pathogenic immune cells is thought to be the dominant mode of action\textsuperscript{50}.

The ability of lymphocytes to migrate between secondary lymphoid organs and the respective target tissue, using the lymphatic and circulatory systems, is instrumental for raising an adaptive immune response. This is not only relevant for primary immune defence but also for T cell- and/or B cell-mediated autoimmune conditions such as MS, where myelin-reactive lymphocytes undergo crucial activation steps in lymph nodes prior to their transit to the CNS\textsuperscript{51–53}. Migration of lymphocytes critically depends on sphingosine 1-phosphate (S1P) receptors for lymph node egress\textsuperscript{54} and on α4β1 integrins for transit to the CNS\textsuperscript{55}. These insights paved the way for the clinical successes of the anti-α4β1 antibody natalizumab\textsuperscript{56} and the S1P receptor modulators fingolimod, ozanimod, ponesimod and siponimod, which are highly efficacious therapies for relapsing variants of MS\textsuperscript{46}. Of note, siponimod was also effective in secondary progressive forms of MS, possibly independently of its anti-inflammatory activity\textsuperscript{57}, supporting the hypothesized contribution of certain S1P receptors to regenerative processes in the CNS\textsuperscript{58}.

Although these immunotherapies have excelled in controlling inflammation — as reflected in a marked reduction of relapse rates (TABLE 1) — and received regulatory approval, they generally fail to halt disease progression and to promote remyelination, with substantial residual disease burden even with treatment. The beneficial therapeutic effects of B cell-depleting agents and siponimod in progressive MS were mainly confined to a subset of patients with potentially active inflammation, visualized by MRI and/or with superimposed relapses\textsuperscript{59}. Furthermore, those therapeutic effects were rather short-lived and limited\textsuperscript{56}. Decelerating the insidious neurodegenerative process, especially in the progressive forms of disease, is even more challenging\textsuperscript{57}. Hence, there is an urgent need for future research to re-evaluate disease pathophysiology beyond autoimmune inflammation to identify novel therapeutic targets that counteract the pathobiological consequences of the chronic stages of the disease.

Oligodendrocytes revisited

In recent years, the focus of MS research has expanded beyond immune cells and recognized the contributions of multiple glial cell types to the development, progression and amelioration of the disease. Oligodendrocytes are specialized glial cells that synthesize myelin sheaths, enable saltatory conduction and provide metabolic support to neurons.

Inflammation regularly damages oligodendrocytes, resulting in demyelination and, consequently, axonal loss\textsuperscript{60}. To effect remyelination, microglia and macrophages must first clear the damaged myelin\textsuperscript{61}, a process that is enhanced by activation of the triggering receptor expressed on myeloid cells 2 (TREM2)\textsuperscript{62}. Next, oligodendrocyte progenitor/precursor cells (OPCs) need to be recruited to the zone of myelin loss and undergo further differentiation and maturation to become fully competent myelin-producing oligodendrocytes\textsuperscript{63,64}. However, the differentiation process from OPC to mature myelin-producing oligodendrocyte is impaired in MS lesions owing to the inflammatory microenvironment and the presence of an array of inhibitory molecules, which might cause inefficient remyelination\textsuperscript{65}. Such an
| Drug                        | Study name | Disease course | Design                                      | Primary outcome parameters                                                                                                                                                                                                 | Ref. |
|-----------------------------|------------|----------------|---------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| IFNβ1B subcutaneous        | --         | RRMS           | Phase III double-blind RCT                  | Relapse rate reduced by 7% (for 1.6 MIU) and by 33% (for 8 MIU)                                                                                                      | 304  |
| Copolymer 1 (predecessor of glatiramer acetate) | --         | RRMS           | Phase III double-blind RCT                  | Relapse rate reduced by 29%                                                                                                                                                                                                     | 305  |
| IFNβ1A intramuscular       | MSCRG      | RRMS           | Phase III double-blind RCT                  | Proportion of patients with disability progression (≥1.0 point on EDSS) at the end of 104-week study time was reduced by 37%                                                                                   | 306  |
| IFNβ1A subcutaneous        | PRISMS     | RRMS           | Phase III double-blind RCT                  | Number of relapses during the study reduced by 27% (22 µg) and 33% (44 µg)                                                                                           | 307  |
| Mitoxantrone               | MIMS       | RRMS or SPMS   | Phase III double-blind RCT                  | Composite of five clinical measures (change in EDSS score, ambulation index and neurological status at 24 months; number of treated relapses; time to first treated relapse) reduced by 30% | 308  |
| Natalizumab                | AFFIRM     | RRMS           | Phase III double-blind RCT                  | Rate of clinical relapse at 1 year reduced by 68%                                                                                                               | 309  |
| Fingolimod                 | FREEDOMS   | RRMS           | Phase III double-blind RCT                  | Annualized relapse rate reduced by 60% (1.25 mg) and 55% (0.5 mg)                                                                                          | 310  |
| Fingolimod                 | TRANSFORMS | RRMS           | Phase III double-blind, active-comparator RCT | Annualized relapse rate reduced by 39% (1.25 mg) and 52% (0.5 mg)                                                                                               | 311  |
| Fingolimod                 | FREEDOMS II| RRMS           | Phase III double-blind RCT                  | Annualized relapse rate reduced by 50% with (1.25 mg) and 48% (0.5 mg)                                                                                          | 312  |
| Cladribine                 | CLARITY    | RRMS           | Phase III double-blind RCT                  | Relapse rate at 96 weeks reduced by 58% (3.5 mg/kg) and 55% (5.25 mg/kg)                                                                                       | 313  |
| Teriflunomide              | TEMSO      | RRMS           | Phase III double-blind RCT                  | Annualized relapse rate reduced by 31% (7 mg) and 32% (14 mg)                                                                                                    | 314  |
| Teriflunomide              | TOPIC      | CIS            | Phase III double-blind RCT                  | Time to relapse (conversion to clinically definite MS) reduced by 37.2% (7 mg) and 42.6% (14 mg)                                                                      | 315  |
| Teriflunomide              | TOWER      | RRMS           | Phase III double-blind RCT                  | Annualized relapse rate reduced by 22% (7 mg) and 36% (14 mg)                                                                                                    | 316  |
| Dimethyl fumarate          | CONFIRM    | RRMS           | Phase III double-blind, active-comparator RCT | Annualized relapse rate reduced by 44% (twice daily dimethyl fumarate), by 51% (thrice daily dimethyl fumarate) and by 29% (glatiramer acetate) | 317  |
| Dimethyl fumarate          | DEFINE     | RRMS           | Phase III double-blind RCT                  | Proportion of patients who had relapsed by 2 years reduced by 41% (twice daily dimethyl fumarate) and 43% (thrice daily dimethyl fumarate)                     | 318  |
| Alemtuzumab                | CAMMS223   | RRMS           | Phase II double-blind, active-comparator RCT | Proportion of patients with sustained accumulation of disability (≥1.0 point on EDSS) reduced by 66% Relapse rate reduced by 72% | 319  |
| Alemtuzumab                | CARE-MS I  | RRMS           | Phase III double-blind, active-comparator RCT | Relapse rate reduced by 55% Proportion of patients with sustained accumulation of disability (≥1.0 point on EDSS) reduced by 27% | 320  |
| Siponimod                  | EXPAND     | SPMS           | Phase III double-blind RCT                  | Proportion of patients with 3-month confirmed disability progression reduced by 21%                                                                                 | 321  |
| Ocrelizumab                | OPERA      | RMS            | Phase III double-blind, active-comparator RCT | Annualized relapse rate over 2 years reduced by 47%                                                                                                               | 322  |
| Ocrelizumab                | ORATORIO   | PPMS           | Phase III double-blind RCT                  | Percentage of patients with disability progression confirmed at 12 weeks in a time-to-event analysis reduced by 16%                                                | 323  |
| Ozanimod                   | SUNBEAM    | RMS            | Phase III double-blind, active-comparator RCT | Annualized relapse rate over 1 year reduced by 50% (1.0 mg) and 31% (0.5 mg)                                                                                  | 324  |
| Ponesimod                  | OPTIMUM    | RMS            | Phase III double-blind, active-comparator RCT | Annualized relapse rate over 2 years reduced by 31%                                                                                                               | 325  |

CIS, clinically isolated syndrome; EDSS, expanded disability status scale; IFN, interferon; MIU, million international units; MS, multiple sclerosis; PPMS, primary progressive MS; RCT, randomized controlled trial; RMS, relapsing MS; RRMS, relapsing-remitting MS.
acute inflammatory microenvironment is associated with the presence of reactive oxygen species, which may in turn affect the fate of OPCs. Moreover, in experimental inflammatory demyelination, OPC differentiation is inhibited by effector T cells and IFNγ. This effect is paralleled by an induction of the crucial immunoproteasome subunit PSMB8 (also known as LMP7), which increases MHC class I expression on OPCs, rendering them a more prominent target for the cytotoxic CD8+ T cells that are abundant in MS lesions. We note that induction of immunoproteasomes in OPCs has been observed in human demyelinated MS brain lesions. Recent evidence suggests that resident oligodendrocytes (rather than those differentiated from recently recruited OPCs) are also capable of remyelinating denuded axons.

Various strategies to increase remyelination by forcing the differentiation of OPCs to mature oligodendrocytes are currently being researched. For example, recombinant galectin 3, which regulates basic cellular functions, can promote differentiation of OPCs to oligodendrocytes by activating AKT kinase and suppressing the extracellular-signal-regulated kinase 1 (ERK1) and ERK2. In addition, inhibiting the ERK1–AMP-activated protein kinase (AMPK) pathway enhances oligodendrocyte generation and thereby promotes remyelination in EAE and drug-induced demyelination models.

Usually, the oligodendrocyte cell lineage is subclassified into oligodendrocytes and OPCs, but there is evidence for greater heterogeneity of the oligodendrocyte cell population in MS. By performing single-nucleus RNA sequencing in white matter areas of post-mortem MS brains, Jäkel et al. demonstrated an increased heterogeneity of oligodendrocytes in MS lesions and identified altered sub-clusters within normal-appearing white matter, indicating that MS is a more diffuse disease than foci suggest. Following this approach, a recent single-cell transcriptomic analysis of oligodendrocytes from the spinal cord of EAE mice and human MS brain samples revealed that oligodendrocyte lineage sub-clusters express genes involved in antigen processing and presentation, highlighting a potential alternative role of oligodendrocytes in the context of inflammatory disease.

We further note that in early EAE axonal damage may precede demyelination, suggesting that loss of metabolic support from oligodendrocytes may cause demyelination. Human oligodendrocytes and OPCs use aerobic glycolysis to maintain cell function and the biosynthesis of myelin. Activation of the oligodendroglial N-methyl-D-aspartate (NMDA) receptor can support axonal energy metabolism by increasing glucose utilization in oligodendrocytes. During times of stress (such as glucose deprivation or hypoxia), oligodendrocytes reduce their glycolytic flux and use ATP for cell survival rather than for myelin production. Inhibiting mitochondrial oxidative phosphorylation in vitro can alter the differentiation of OPCs to oligodendrocytes and thereby affect myelin production. Moreover, differentiation of oligodendrocytes requires reorganization of lipid metabolism, including the biosynthesis of cholesterol and sphingolipids, which are major components of myelin. Using two independent — inflammatory versus toxic — models of demyelination and remyelination, Voskuhl and colleagues showed that genes involved in cholesterol synthesis were upregulated in oligodendrocytes during the remyelination phase. Treatment with an oestrogen receptor-β ligand further increased cholesterol synthesis, indicating a potential target for enhancing remyelination. Furthermore, fatty acid synthase in oligodendrocytes is crucial for the correct lipid composition in myelin, and depletion of fatty acid synthase in OPCs leads to defects in remyelination in a mouse model of demyelinating spinal cord lesions, highlighting fatty acid synthase as another potential target for remyelination.

The initial oligodendrocyte targeting therapies addressed endogenous checkpoints that otherwise inhibit remyelination, such as the leucine-rich repeat and immunoglobulin-like domain-containing protein 1 (LINGO1). Opicinumab, an anti-LINGO1 monoclonal antibody that modulates OPC differentiation to enhance remyelination, was tested in pilot phase II studies in optic neuritis and relapsing-remitting multiple sclerosis (RRMS) (RENEW and SYNERGY trials) and phase IIb studies in optic neuritis and relapsing-remitting RRMS. Both studies failed to meet their respective primary endpoints, but exploratory analyses suggested a potential benefit of this approach: in RRMS, participants of younger age and short disease duration responded better to opicinumab. This highlights the potential of oligodendrocyte-orientated therapies in the future. However, a recent phase II study (AFFINITY) evaluating opicinumab as an add-on therapy to standard immunotherapy failed to reach the primary end point of improvement of the disability in comparison to placebo. Remyelination starts immediately after the onset of inflammatory demyelination, leading to the speculation that remyelination therapies need to be administered shortly after demyelination has occurred to improve the remyelination capacities of dysregulated inflammatory oligodendrocyte/OPC subtypes. Thus, remyelination-promoting therapies such as opicinumab may fail if they do not target the inflammatory oligodendrocyte/OPC phenotype in the early remyelination phase.

Another remyelination approach recently explored in experimental and clinical settings targets the histamine and muscarine receptor systems. The histamine H1 receptor antagonist clemastine, established as an allergy therapy in clinical practice, was identified in an unbiased drug repurposing screen for compounds with remyelination capacities. Clemastine, previously shown to induce differentiation of OPCs and promote remyelination in experimental demyelination models, was further examined in a pilot trial in optic neuritis, with confirmatory but rather modest clinical effects. We note that clemastine antagonizes not only the H1 receptor, but also muscarinic receptors in a non-selective manner. Another nonselective muscarinic receptor antagonist, benzatropine, enhanced remyelination in experimental rodent models. In-depth analysis showed that signalling via the muscarinic M1 receptor is pivotal for inhibition of efficient remyelination by...
Fig. 2 | Role of oligodendrocytes in MS. Oligodendrocytes differentiate from neuronal stem cells to oligodendrocyte progenitor cells (OPCs) and pre-oligodendrocytes, a process orchestrated by several pathways and factors (oligodendrocyte transcription factor 2 (OLIG2), homeobox protein NKX2.2, SRY-box transcription factor 10 (SOX10), myelin regulatory factor (MYRF), SOX5/6, HES family bHLH transcription factor 5 (HES5), inhibitor of DNA binding 2 (ID2) and ID4). The different development stages of oligodendrocytes are typified by various oligodendroglial lineage markers (OPC: platelet-derived growth factor receptor-α (PDGFRα) and neuron-glial antigen 2 (NG2); pre-oligodendrocytes: 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNPase) and oligodendrocyte marker 4 (O4); oligodendrocytes: galactocerebroside (GalC), CNPase, myelin-associated glycoprotein (MAG), myelin basic protein (MBP) and proteolipid protein (PLP)). In multiple sclerosis (MS), the autoimmune attack (orange arrow) drives inflammatory demyelination, which is characterized by astrocyte activation and microglia recruitment/activation. This leads to demyelination and axonal damage and is associated with astroglial proliferation and oligodendrocyte depletion, resulting in a glial scar (blue arrow). Strategies to modulate OPC differentiation to enhance remyelination (leucine-rich repeat and immunoglobin-like domain-containing protein 1 (LINGO1) and oxidative phosphorylation (OXPHOS)) are shown. To force successful regeneration (grey arrow), potential pathways are metabolic support (enhancement of glucose utilization or fatty acid and cholesterol synthesis) or promotion of OPC differentiation to oligodendrocyte (galectin 3).
pathophysiological neuroinflammatory process. In neurological diseases, but especially in MS, in which recovery may be a promising protective strategy in several instances. Whether global remyelination is able to restore the network of functions of the CNS in patients with MS remains an open question.

**The blood–brain barrier as an early target**

The BBB consists of specialized endothelial cells (ECs), which communicate with other cells (including astrocytes, pericytes, neurons, smooth muscle cells, microglia and other immune cells) of the CNS to form the neurovascular unit. The breakdown of the BBB is an early hallmark and key pathophysiological event in MS and can be visualized by the leakage of contrast agents during MRI. Gadolinium enhancement is observed in active lesions but other — more sophisticated — MRI measures demonstrate subtle BBB disruption in normal-appearing white matter. This suggests that covert BBB disruption may precede neuroinflammatory processes.

RNA sequencing in mouse brain ECs revealed that during BBB dysfunction a similar gene expression pattern occurred in different disease models (stroke, multiple sclerosis, traumatic brain injury and seizure) when the BBB was at its most dysfunctional. Within those disease models EAE showed the most unique changes in brain ECs, with a specific gene expression pattern — including leukocyte adhesion molecules and histocompatibility loci, as well as interferon-induced, interleukin and complement pathway genes. The integrity of the BBB is tightly regulated, and preserving this integrity may be a promising protective strategy in several neurological diseases, but especially in MS, in which BBB disturbance actively participates in initiating the pathophysiological neuroinflammatory process.

In MS, autoreactive leukocytes enter the CNS after peripheral activation of cellular migration molecules together with chemokine and adhesion receptors (Fig. 3). The functional phenotype of infiltrating immune cells depends on where priming occurred; for example, cells can be primed in the skin or gut. Brain ECs control the transmigration of leukocytes by expressing adhesion molecules and producing chemokines. Selectins capture and induce rolling of immune cells; however, their absence or pharmacological blockade had no impact on EAE development in mice. Next, firm adhesion is mediated by adhesion receptors on ECs and their counterpart ligands (such as integrins) on leukocytes. Natalizumab, an antibody that targets the leukocyte ligand α4β1 integrin and is approved for the treatment of RRMS, impairs the adhesion of leukocytes to vascular cell adhesion molecule 1 (VCAM1) on brain ECs. However, treatment with natalizumab may be associated with side effects that are probably due to widespread functions of α4 integrin in haematopoietic cells, indicating a need for alternative therapeutic strategies that inhibit only the migration of pathogenic lymphocytes. For this purpose, other approaches to brain-specific inhibition of leukocyte-endothelial interaction could be a promising strategy. Additional brain EC-specific adhesion molecules, such as activated leukocyte cell adhesion molecule (ALCAM; also known as CD166), have been implicated in EAE and MS pathogenesis and may be useful therapeutic targets. However, leukocytes can bypass the blockade of adhesion receptor–ligand interaction by using alternative adhesion molecules. Therefore, strategies targeting transmigration directly by inhibition of cell adhesion molecule biosynthesis/expression on ECs or indirectly through reduction of the inflammatory EC phenotype (thereby reducing adhesiveness), might circumvent this shortcoming.

Different pathways have been identified that control the morphology and adhesive capacity of ECs. For example, the kallikrein–kinin system regulates the expression of VCAM1 and intercellular cell adhesion molecule 1 (ICAM1) on brain ECs via a PAR2-receptor-mediated pathway. Further unexpected targets might also be involved in the regulation of adhesive capacity, given that the potassium channel TREK1 was shown to modulate VCAM1 and ICAM1 expression on brain ECs.

The WNT–β-catenin pathway, known to be involved in BBB formation and maintenance, is activated in brain ECs in human MS lesions and the EAE model. Inhibition of the endothelial WNT–β-catenin pathway prior to disease onset leads to a more severe disease course in EAE, accompanied by BBB disruption and increased immune cell infiltration into the CNS, indicating that endothelial WNT–β-catenin pathway reactivation could be a strategy to maintain BBB integrity in inflammatory conditions. However, WNT activation in perivascular OPCs in white matter lesions leads to secretion of WIF1, which counteracts the effects of WNT ligands in ECs and leads to endothelial dysfunction. Furthermore, inhibition of WNT signalling in oligodendrocytes leads to regenerative myelination, perhaps because this pathway is involved in oligodendrocyte maturation and myelination. Overall, pharmacological enhancement of WNT signalling may be considered as a strategy to preserve BBB integrity, but those therapies need to be highly cell-specific to avoid potential side effects.

Another potential target, liver X receptor-α (LXRα), is a nuclear receptor involved in cholesterol and lipid metabolism. Endothelial LXRα is involved in...
maintaining BBB integrity. EC-specific knockdown of LXRα increases BBB permeability in vitro and in vivo and is associated with reduced tight junctions, increased VCAM1 expression and leukocyte infiltration. Moreover, EC-specific LXRα-deficient mice show exaggerated disease progression in the EAE model, indicating that LXRα could be a potential new target for improving BBB function117.

Adherent and junction molecules stabilize and tighten the BBB. The platelet/endothelial cell adhesion molecule 1 (PECAM1) maintains EC integrity and is abundant at cell–cell junctions118. PECAM1-deficient mice exhibit early onset of symptoms and leukocyte infiltration in EAE119. In vitro, PECAM1 stabilizes BBB integrity and may thereby have neuroprotective functions in neuroinflammatory conditions120. A leukocyte transmigration inhibitor (trioxotetrahydropyrimidine scaffold, compound 12) improved the clinical score in EAE without any toxic side effects. This compound blocks PECAM1 in an in silico model, although the observed effects may have been transmitted by a different mechanism121. Therefore, more studies are needed to evaluate the potential role of PECAM1 in MS.

BBB dysfunction in MS is associated with decreased levels of tight junction proteins. Downregulation of claudin 5 correlates with BBB breakdown in EAE, and recombinant expression of claudin 5 protects brain microvascular ECs from vascular endothelial cell growth factor-α (VEGFa)-induced barrier dysfunction122. In EAE, a subpopulation of claudin 5-positive leukocytes was observed in close apposition to inflamed vessels. Claudin 5 may be transferred via extracellular vesicles from ECs to leukocytes to facilitate transendothelial leukocyte migration via claudin 5 bridges in EAE123. In addition, peripheral blood leukocytes in MS patients experiencing a clinical relapse show increased claudin 5 levels124. Additional pathways (including PDGFβ–PDGFβR, TGFβ–TGFβR, SHH–PTC1, ANG1–TIE2, ANG II–AT1 and APOE–LRP1) have been implicated in tight junction formation and may therefore be interesting targets for further research in MS therapy125–128.

Blood–brain barrier

Fig. 3 | Blood–brain barrier disruption in MS. In physiological conditions, blood–brain barrier integrity is tightly regulated. In multiple sclerosis (MS), the tight barrier can be dysfunctional, leading to increased leukocyte recruitment and transmigration. In addition, autoreactive T cells can enter the CNS through peripheral activation of cellular locomotion molecules, together with chemokine and adhesion receptors. T cell trans-endothelial migration proceeds in several steps (steps 1 to 5) that are controlled by various adhesion molecules (selectins, lymphocyte function-associated antigen 1 (LFA1) and α4β1 integrin) and their receptors (selectin ligands, intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1)) expressed by endothelial cells (ECs). Furthermore, tissue-resident macrophages can secrete factors (cytokines, chemokines and growth factors) that lead to EC activation, characterized by increased expression of selectin and adhesion molecules. ALCAM, activated leukocyte cell adhesion molecule; ANG, angiopoietin; CCL2, CC-chemokine ligand 2; CCL21, CC-chemokine ligand 21; CXCL12, CXC-chemokine ligand 12; LXRα, liver X receptor-α; ICAM, melanoma cell adhesion molecule; MMP2/9, matrix metalloproteinase 2/9; PSGL1, P-selectin glycoprotein ligand 1; TGFβ, transforming growth factor-β; TIE2, tyrosine-protein kinase receptor TIE2; VAP1, vascular adhesion protein 1.
To take the final step into the CNS parenchyma, leukocytes have to breach the endothelial basement membrane and the glia limitans. Matrix metalloproteinases (MMPs) are essential for this step because they digest tight junction and basal membrane proteins. MMP9 protein and RNA levels are increased in serum, mononuclear cells and cerebrospinal fluid (CSF) and correlate with disease progression. In addition, MMP activity, detected by MMP inhibitor–positron emission tomography (MMPi-PET), is a unique feature of early MS lesions. Young mice deficient in MMP9 are relatively resistant to EAE induction.

In recent decades, pharmacological MMP inhibitors have showed efficacy in experimental animal models but have failed in clinical trials, with limited beneficial effects and serious adverse events. Since MMPs are involved in several important biological pathways (including tissue morphogenesis, angiogenesis and cell migration), inhibiting all MMP family members leads to adverse effects. Treatment with more-specific inhibitors, such as triple-helical peptide inhibitors, which target MMP9 and MMP2, reduces EAE severity. Furthermore, the monoclonal anti-MMP9 antibody ancaliximab has been tested in initial phase I clinical trials for other autoimmune diseases such as rheumatoid arthritis or ulcerative colitis and was found to be safe and well tolerated, so MMP9 inhibition may be safe in MS.

Some of the effects of IFNβ, a standard treatment for MS, may be through MMP9-mediated BBB regulation, because IFNβ downregulates MMP9 expression to reduce the migratory capacity of immune cells. Chemokines produced by ECs are involved in all stages of the transmigratory process. Brain ECs induce firm adhesion of T cells in EAE and MS through CC-chemokine ligand 19 (CCL19) and CCL21. CXC-chemokine ligand 12 (CXCL12) also has a role in EAE and MS pathogenesis as it mediates T cell arrest on brain ECs and the basolateral release of inflammatory cells. Chemokine-targeted therapies can be exploited to regulate dysfunctional chemokine production in MS.

Other cells of the neurovascular unit critically influence BBB integrity and might therefore be therapeutically targeted. Pericytes ensheathe the endothelial monolayer of the BBB and regulate BBB function. Pericytes contribute to MS pathogenesis by expressing adhesive molecules, producing pro-inflammatory mediators (including cytokines, chemokines and MMPs), presenting antigens and producing reactive oxygen species. Therefore, pericytes might be interesting therapeutic targets in MS; however, specifically targeting pericytes is challenging. Lipid and protein carriers with pericyte-targeting motifs have been developed and may be instrumental for future pericyte-directed therapies.

Cell-based strategies have also been proposed to treat BBB breakdown. In rodent models, systemically administered mesenchymal stem cells reduce leukocyte transmigration and regulate the production of MMPs, reactive oxygen species and pro-inflammatory cytokines, as well as stabilizing the cellular components of the neurovascular unit. Small-scale clinical trials in MS supported the feasibility and safety of mesenchymal stem cell administration. However, larger studies are needed to evaluate the efficacy of those approaches.

Numerous interactions have been demonstrated between the gut microbiome and the BBB. Metabolic products, cytokines or other immune-active substances can alter BBB integrity, transport rates and phenotypes of barrier cells. Further, certain bacterial factors promote CNS penetration of T cells. Thus, the gut microbiome might be another target to influence BBB function and immune cell infiltration to the CNS in the context of MS.

In summary, the preservation or restoration of BBB integrity is a promising new target for MS therapy and may be used to treat additional CNS disorders. However, most approaches are far from clinical development and targeted therapies are needed to specifically address dysregulation of brain ECs or other components of the neurovascular unit without affecting physiological function. The benefits of targeting BBB integrity are mostly confined to early stages of MS pathogenesis, whereas chronic neuroinflammation and subsequent neurodegeneration are unlikely to be affected by this treatment strategy. Furthermore, alternative routes to the CNS such as the plexus epithelium might circumvent BBB-targeting therapies and need to be considered in the clinical development of new treatment strategies.

Is multiple sclerosis a metabolic disease?

New technologies (such as metabolomics) have provided useful insights into the cellular metabolism of cancer and inflammatory diseases. In this section, we focus on metabolic alterations occurring in patients with MS and describe the metabolic profile of cells — T cells and neurons — that are primarily involved in MS pathogenesis.

Metabolites in MS. Metabolites are intermediate or end products of numerous physiological and pathological cellular processes and can be detected within cells as well as biological samples available in clinical practice — specifically, CSF, serum, urine and tissue. Several metabolic changes are reported in MS (Supplementary Table). Here, we focus on metabolites that have a role in MS disease progression and could unveil new biomarkers or therapeutic targets.

Several studies report alterations in metabolite levels of amino acids in MS. The amino acid glutamate is a neurotransmitter, and excessive levels are excitotoxic. Glutamate and glutamine levels are elevated in the plasma of patients with RRMS (and other neurologic diseases), and glutamate concentration correlates with disease severity in RRMS. In addition, levels of branched-chain amino acids (leucine, isoleucine and valine), which are substrates for glutamate synthesis and have an important role in transporting amino acids through the BBB, are decreased in patients with RRMS.

Recently, Fitzgerald et al. identified abnormalities in aromatic amino acid (AAA) metabolites using a multi-omic approach in patients with MS. A reduced quantity of AAA metabolites correlated with higher disability,
and altered AAA metabolism was found in CSF- and serum-derived monocytes of patients with MS. These AAA metabolites may come from the gut microbiota\textsuperscript{154}. Levels of butyrate- or indolelactate-producing bacteria are reduced in patients with MS\textsuperscript{155}. Indolelactate is an intermediate product of tryptophan degradation in bacteria and tryptophan metabolism is involved in inflammatory processes. High levels of microbiota-derived indolelactate are also associated with a lower risk of developing paediatric MS\textsuperscript{156}. Butyrate influenced T cell differentiation and suppressed demyelination in vivo\textsuperscript{157}. However, the gut microbiome profile did not differ among the different forms of MS or in response to treatment with DMT\textsuperscript{155}.

In accordance with this altered gut microbiota, bile acid metabolism is altered in patients with MS\textsuperscript{154,156,164}. Endogenous bile acid supplementation is neuroprotective, ameliorates disease severity in an EAE model and is currently being evaluated in a phase 1 clinical trial\textsuperscript{158}. Although not within the scope of this Review, there is an evolving understanding of the interaction between the gut microbiome and the pathophysiology of MS; influencing the gut microbiota might be another therapeutic avenue (reviewed elsewhere\textsuperscript{160–163}).

Modulating the metabolism of the AAA tryptophan, which is reduced in the serum and CSF of patients with MS\textsuperscript{154,164,165}, could have therapeutic value. In vivo models showed reduced levels of tryptophan metabolites on both sides of the BBB (cortex and serum) during demyelination\textsuperscript{166}. Furthermore, the ratio of tryptophan to kynurenine, a key tryptophan metabolite, in urine negatively correlates with the disability score of patients with RRMS\textsuperscript{167}. Kynurenine can be metabolized to either quinolinic acid, which is neurotoxic, or kynurenic acid,
which is neuroprotective. The balance of these neurotoxic and neuroprotective kynurenic metabolites is disturbed during MS progression. Increased levels of kynurenic acid were observed in patients with RRMS but not in those with SPMS or PPMS, whereas neurotoxic quinolinic acid concentrations were progressively raised in both SPMS and PPMS. The upregulated levels of kynurenic acid may compensate for quinolinic acid-induced excitotoxicity in the early stage of the disease, whereas neurotoxicity dominates in progressive stages. Interestingly, quinolinic acid levels are positively associated with the gut microbiota, which is known to be altered in patients with MS.

Lipid metabolism is also altered in MS: sphingolipid levels are decreased and phospholipid levels are increased in active MS lesions. Levels of phospholipids, particularly lysophosphatidylcholine (LPC), are also elevated in the CSF of patients with MS. LPC is cleaved from a major component of the membrane, phosphatidylinositol, by phospholipase A2 (PLA2). PLA2 activity is associated with neuroinflammatory diseases and dysfunctional BBB. Thus, the high LPC levels in the CSF of patients with MS indicate augmented PLA2 activity, which may be important for initiating membrane breakdown. Interestingly, inhibition of PLA2 protects mice from acute relapse in the EAE model, preventing membrane breakdown and reducing potential pathological effects of LPC and other phospholipid metabolites.

When considered together, these studies indicate a distinct metabolic profile in MS. Differentiating between the origin of the samples (CSF versus blood) and the knowledge that some metabolites, such as lactate and fructose, cannot pass the BBB is important for future analysis. Furthermore, the use of different techniques (nuclear magnetic resonance (NMR) versus mass spectrometry) and differences in sample handling and other factors (such as storage conditions) can limit comparability between metabolic studies. Further investigations with larger cohorts and standardized methods are needed to validate the metabolic signature of MS derived from blood or CSF.

**T cell metabolism.** T cells are highly adaptive and require energy and metabolites for proliferation, activation and differentiation into specific cell subsets. To fulfill these manifold functions, their metabolism adapts. Master transcription factors and immune signals orchestrate T cell fate, and cell metabolism can also dictate this decision.

Quiescent T cells fuel their energy demand through mitochondrial respiration and fatty acid oxidation. In contrast, proliferating T cells have a dynamic metabolism and rely mainly on glycolysis, which provides energy quickly, and increase glucose influx by increasing expression of the glucose transporter GLUT1 (REF. (18)).

Activated CD4+ T cells differentiate into effector CD4+ T cells, including TH1 and TH17 cells, and regulatory T cells. TH17 cells can induce MS-like pathology in experimental models, and these cells are the first encephalitogenic T cells to infiltrate the CNS, which leads to secondary immune cell infiltration. In contrast, Treg cells suppress the activity of TH17 and TH1 cells and thereby reduce neuroinflammation in MS.

The metabolism of all of these cells could be targets for therapies. The metabolism of CD4+ T cells is dysregulated in MS, and recent studies attempted to decipher their metabolic properties to identify new potential drug targets. In peripheral immune cells from patients with RRMS, glycolysis and oxidative phosphorylation were impaired during T cell activation. However, the study did not distinguish between T cell subsets, and the patient cohort was small. In another study, CD4+ T cells activated in vitro from patients with RRMS showed increased oxidative phosphorylation and glycolysis if isolated from patients during relapses, but not from those in remission. Inhibiting the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), which affects complex III of the respiratory chain, reduced the number of high-affinity T cells produced in patients with RRMS, probably by altering the metabolic properties of these cells during relapse.

Cell metabolism can determine T cell fate, making it a favourable target for counteracting the deregulated T cell balance in MS. T cell activation is accompanied by a rapid increase in mitochondrial oxidative phosphorylation during lineage specification towards pathogenic TH17 cells. Differentiated TH17 cells mainly rely on glycolysis and fatty acid synthesis (FAS) to fulfill their energy and biosynthesis demands. Inhibiting glycolysis with either 2-deoxy-D-glucose or inhibitors of pyruvate kinase slows EAE progression. Moreover, dimethylfumarate, a drug approved for the treatment of relapsing MS, acts at least in part by blocking glycolysis in TH17 cells. In addition, inhibiting the glucose transporter GLUT1 suppresses TH17 differentiation and increases Treg cell induction. Furthermore, blockade of acetyl-CoA carboxylase 1 (ACCI), which catalyses the first step in FAS, decreases the TH17 cell population and promotes the development of Treg cells, and thus attenuates inflammation in the EAE model. TH17 cells also depend on glutaminolysis for energy and upregulate glutaminase 1 (GLS1). Genetic disruption of GLS1 or pharmacological inhibition of either GLS1 or the glutaminolytic pathway enzyme glutamic oxaloacetic transaminase 1 (GOT1) reduces initial T cell proliferation and impairs TH17 differentiation, and thereby ameliorates disease progression in EAE. Moreover, further, mice deficient in the neutral amino acid transporter B0 (also known as ASCT2), a Na+-dependent transporter that regulates glutamine uptake upon T cell activation, were protected from EAE initiation via impaired TH1 and TH17 cell induction.

Treg cells and T memory cells have historically been thought to use fatty acid oxidation (FAO) to generate reducing agents for oxidative phosphorylation and intermediates for the TCA cycle to sustain their energy needs, but the role of FAO in some T cell subsets has been called into question. The rate-limiting enzyme for FAO is carnitine palmitoyl-transferase 1a (CPT1A), which transports fatty acids into the mitochondria, where they undergo β-oxidation. Inhibition of mTOR

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**T helper 1 cells (TH1 cells).** A subgroup of T helper cells (also known as CD4+ cells) that is mainly involved in the cell-mediated immune response against intracellular pathogens such as bacteria by maximizing the efficacy of macrophages and cytotoxic T cells. The main effector cytokines of TH1 cells are IFNγ and IL-2.

**Treg cells**

A subgroup of T helper cells that is involved in the humoral immune system. Treg cells secrete IL-4 and IL-10 (among others), are involved in the recognition of extracellular pathogens and activate the B cell-mediated antibody response.

**TH17 cells**

A subgroup of T helper cells that is developmentally distinct from TH1 and TH2 cells and is defined by production of IL-17. TH17 cells are involved in host defence against extracellular pathogens, but also contribute to the pathogenesis of immune-mediated diseases such as MS.

**Regulatory T (Treg) cells**

A subpopulation of CD4+ T cells, also known as suppressor T cells, characterized by the expression of CD4 and CD25. Treg cells have a critical role in preventing autoimmunity by controlling the immune response to self-antigens and can inhibit T cell proliferation and cytokine production.
or activation of AMP-activated protein kinase (AMPK) pathways can inhibit glycolysis in Th17 cells and favour T cell differentiation to Treg cells with increased CPT1A expression and lipid oxidation205,206. Treatment with the pharmacological CPT1A inhibitor etomoxir in vitro did not alter Th17 differentiation but suppressed Treg formation207. Counterintuitively, mice with a specific mutation in CPT1A, which is associated with low susceptibility to MS in the Inuit population and reduced CPT1a activity, have reduced disease severity in EAE compared to wild-type mice204. Inhibiting FAO via etomoxir reduced CNS inflammation and demyelination in EAE205,206, indicating that overall reduction of CPT1A activity (and consequently FAO) can ameliorate neuroinflammation.

Mechanistically, etomoxir induces apoptosis of activated myelin oligodendrocyte glycoprotein (MOG)-specific T cells (CD8+) in vitro, and thereby reduces cytokine production. However, Raud et al. demonstrated that etomoxir in higher dose presents an off-target effect with inhibition of T cell proliferation and differentiation207. Recent studies in mice lacking CPT1A in T cells question the role of FAO in Tmem and Treg cells, given that they showed that CPT1A is largely dispensable for the formation of Tmem cell or Treg cell differentiation202, suggesting an alternative mechanism for the effects of etomoxir in the EAE model.

Overall, fatty acid metabolism represents a promising target to counteract neuroinflammation. However, it is questionable whether an altered T cell metabolism underlies the observed effects in vivo and more studies are needed to clarify the role of FAO in contributing cell types in the pathophysiology of MS.

Differentiation into pro- and anti-inflammatory T cell subsets is also influenced by epigenetic mechanisms, including chromatin modelling. Generally, the activity of chromatin-modifying enzymes is regulated by the availability of substrates or co-factors. Methionine is essential for synthesizing the methyl donor S-adenosylmethionine (SAM), which is a cofactor for regulating gene expression in T cells. Dietary methionine reduction slowed EAE disease onset and progression via impaired Th17 cell proliferation208. Furthermore, dietary serine restriction can influence pathogen-driven T cell expansion in vivo as serine supplies glycine and one-carbon units for de novo nucleotide biosynthesis in proliferating T cells209. Consistent with this observation, T cells show altered serine metabolic pathways in the murine EAE model205. T cell differentiation can be influenced by post-translational modification via citrullination, a conversion of peptide arginine into peptidyl citrulline, which is catalysed by the peptidylarginine deiminases, including PAD2 (REF 211). Inhibition of PAD2-mediated citrullination can attenuate the Th17 response, and pharmacological peptidylarginine deiminase inhibitors prevent disease progression in EAE212,213.

A further epigenetic mechanism is involved in controlling T cell fate decisions. (Aminoxy)-acetic acid (AOA) reprograms Th17 differentiation towards Treg cells. AOA inhibits GOT1, which transfers glutamate to α-ketoglutarate214. This decreased levels of 2-hydroxyglutarate, which usually inhibits transcription of FOXP3, an essential transcription factor for Treg cell determination. Since the balance of Th17 and Treg cells is important in MS, the inhibition of GOT1 via AOA also mitigated EAE pathogenesis by reducing the proportion of CNS-infiltrating Th17 cells by more than 70%215.

In summary, manipulating the metabolic program or the substrates of T cell metabolism may be a new tool to develop therapeutic strategies that orchestrate T cell differentiation and thereby influence their function in MS.

Neuronal metabolism. Neuronal metabolism. Neuronal metabolism. Neuronal metabolism.

Neurodegeneration is a major challenge in the therapeutic management of MS. In healthy individuals, neurons require large amounts of ATP to sustain membrane potential and mitochondrial homeostasis over long-ranging axonal projections. To sustain the production of ATP while minimizing oxidative stress, neurons metabolize lactate through oxidative metabolism. Lactate is shuttled via glucose to the pentose phosphate pathway, resulting in the production of NADPH, an essential cofactor for the synthesis of glutathione — an important antioxidant molecule in the CNS. Accordingly, the glycolysis rate in healthy neurons is low and the activation of glycolysis leads to neuronal death through oxidative stress, potentially via a reduced availability of glucose for the pentose phosphate pathway216.

To meet the requirements for ATP and reducing agents, neurons rely on the support of glia to provide metabolic intermediates such as lactate via the astrocyte–neuron lactate shuttle213 (FIG. 4c).

In MS, neuronal death results from an energy imbalance: increased energy demand coupled with dysfunctional energy supply. Single-nucleus RNA sequencing in cortical and subcortical white matter lesions of human brain samples of patients with MS revealed that upper-layer excitatory neurons in MS lesions upregulate genes involved in oxidative stress, mitochondrial dysfunction and cell death217. From a metabolic perspective, MS neurons exhibit reduced oxidative phosphorylation, further indicating mitochondrial dysregulation217. The increased energy demand is caused by sodium/potassium pumps, which need to increase their activity in order to propagate action potentials after the myelin sheath has been damaged218. Energy supply via astrocytes is also affected in MS. During inflammation, astrocytes have a lower activity of hexokinase 2, an enzyme that catalyses the initial step in glycolysis, resulting in reduced glycolysis and impaired lactate release. Pharmacological suppression of pathogenic astrocyte metabolic reprogramming using miglustat, a drug approved for Niemann–Pick disease type C, is beneficial in EAE219.

Neuronal — as well as oligodendroglial — death in MS is also associated with oxidative stress220. Reactive oxygen species are critically involved in neurodegeneration221, particularly during an acute immune attack targeting the myelin sheath222, and the co-factors NADPH and NADH are pivotal for neuronal redox homeostasis. A recent analysis showed decreased NADH levels within the retinas of patients with MS223 and an altered NAD+/NADH ratio in the serum of patients with MS, indicating chronic oxidative stress224. However, it remains to
be determined whether this is a product of a defective defence mechanism or increased production of reactive oxygen species.

In parallel, established concepts of oxidative stress have recently been challenged and expanded, as subtle redox alterations may modulate intracellular signalling cascades and thus the course of autoimmune neuroinflammation. We note that ion imbalance also seems to occur in neurons of patients with MS. Specifically, elevated sodium levels were detected within lesions and high sodium levels were observed in individuals with SPMS and greater disability, indicating an imbalance in ion channelling. Finally, increased intracellular sodium levels can lead to reverse operation of the sodium/calcium exchanger, causing high intracellular calcium levels, resulting in mitochondrial damage and finally axonal degeneration. Thus, manipulating the ion balance might serve as a potential target to counteract mitochondrial damage and prevent neuronal death.

The coagulation system
Several studies have highlighted an important link between the blood coagulation cascade and neuroinflammation. Dysregulation of coagulation factors can contribute to inflammatory neurodegeneration in MS, so these factors may be new therapeutic targets [FIG. 5].

Coagulation factors such as tissue factor (TF) and protein C inhibitor (PCI), which are part of the proximal extrinsic coagulation cascade, are expressed in chronic active plaque samples from patients with MS. Furthermore, protein C plasma levels are associated with neurodegenerative MRI outcomes in MS — specifically, low grey matter volume. In addition, thrombin activity has been shown to precede the onset of neurological signs in an EAE model, and increased thrombin activity was observed at the peak of the course of the disease.

Alterations in proximal parts of the intrinsic coagulation pathway have been implicated in MS pathogenesis. Patients with MS show high plasma levels of factor XII (FXII) during relapse. Pharmacological blockade of FXII leads to reduced susceptibility in an EAE model — an effect that is mediated by a shift in the cytokine profile of dendritic cells that reduces immune activation. In addition, factor X (FX) serum levels are also increased in individuals with MS, and inhibition of FX with rivaroxaban, a clinically approved anticoagulant therapy, can reduce EAE severity.

Inhibition of more distal parts of the coagulation system using hirudin, a thrombin inhibitor, or recombinant activated protein C reduced EAE severity. EAE amelioration via thrombin inhibition is associated with decreased immune cell proliferation and cytokine production. Dabigatran, a clinically approved anticoagulant drug, suppresses the thrombin-induced activation of astrocytes and thereby effectively recovers neurological function and protects against demyelination in EAE. We note that thrombin is a well-known activator of pro-inflammatory proteinase activated receptor 1 (PAR1) signalling, so some of the effects of thrombin inhibition may be mediated through reduced PAR1 activity.

During BBB disruption, the terminal blood coagulation factor fibrinogen is able to enter the CNS and be converted to fibrin, which then activates an immune response. BBB disruption is one of the earliest hallmarks of MS pathology, with fibrin deposition observed throughout the course of the disease and detected in the CSF of patients with MS. Pre-demyelinating lesions demonstrate BBB disruption and increased fibrin deposition with local microglial activation. In progressive MS, fibrin is detected in active and chronic lesions and...
fibrin deposition is associated with neuronal loss. Recently, Ryu et al. developed a monoclonal antibody (5B8) that specifically targets the cryptic fibrin epitope γ377–395 and selectively inhibits fibrin-induced inflammation without altering clotting. Application of this antibody suppressed the innate immune response, oxidative stress, demyelination, and axonal damage and, in aggregate, ameliorated the course of the disease in multiple experimental models of MS.

Plasminogen is converted to plasmin, which cleaves fibrin networks. Interestingly, tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA),
Immune tolerance induction aims to treat multiple sclerosis (MS) at the initial stages of pathogenesis. Various approaches are of interest to induce immune tolerance and can be separated into antigen-specific (labelled 1 to 8) and unspecific tolerance strategies (labelled 10 to 13). The mechanisms (labelled 9) of immune tolerance are driven by tolerogenic antigen-presenting cells (APCs). Application of the different potential auto-pathogen factors can lead to the development of those cells. APC interaction with T cells leads to several changes in the immune response: the induction of regulatory T (Treg) cells; reduction of effector T cells; an increase in exhausted T cells and Treg cells with strong bystander immunosuppression capacity; apoptosis of autoreactive T cells; and anergy in T cells. Ag, antigen; AHR, aryl hydrocarbon receptor; CAR, chimeric antigen receptor; CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; GAL9, galectin 9; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; PD1, programmed cell death 1; PD-L1, programmed cell death 1 ligand; RBC, red blood cell; Treg, T cell regulator; TGFβ, transforming growth factor-β; TIM3, T cell immunoglobulin mucin receptor 3; UCB, umbilical cord blood.

which cleave plasminogen to plasmin, have heightened activity in MS lesions and in the CSF of patients with MS. Deficiency of tPA or uPA leads to an earlier onset and more severe EAE course241,242, but neuronal tPA overexpression failed to alter the disease42. In contrast, treating mice with tPA variant proteins or plasminogen activator inhibitor 1-derived peptide (PAI1-dp) — which block the intrinsic binding of PAI1 and thereby increase the activity of tPA and uPA — significantly ameliorates disease severity in EAE. This effect might be mediated, at least in part, through an increased number of Treg cells and diminished T cell reactivity241,242. Recombinant tPA is used as a thrombolytic agent in ischemic stroke; however, owing to bleeding caused by its catalytic activity, it can be administered only once and is therefore not useful in chronic disease. Other preclinical anticoagulative therapies such as infestin-4, a highly specific FXII inhibitor, can improve disease progression in EAE without compromising haemostasis, as seen in other animal models241,242. To overcome possible bleeding or thrombotic side effects, more-specific therapies are needed that target only the potentially immunomodulatory aspect of the coagulation system.

Tolerance induction

The breakdown of immunological tolerance mechanisms is a hallmark of MS pathogenesis. Autoreactive T cells are eliminated by central tolerance mechanisms in the thymus. However, this process is imperfect and some autoreactive T cells are released into the circulation. Under physiological conditions, those autoreactive cells are controlled by peripheral immune tolerance, which is mainly mediated by Treg cells. In MS, autoreactive T cells and autoantibodies against CNS antigens as well as impaired Treg cell function can be detected and have been implicated as central drivers of pathology240,246.

Therapeutic approaches addressing those pathogenic factors with antigen-specific tolerization (AST) or antigen-unspecific tolerization strategies have already been tested at early stages of clinical development in MS (Fig. 6). The rationale behind AST is to silence and/or remove autoreactive CD4+ T cells that recognize CNS antigens in an HLA-DR-restricted manner. Previous therapeutic approaches focused mainly on myelin proteins such as myelin basic protein (MBP), myelin proteolipid protein (PLP) or MOG, because they are encephalitogenic in EAE and are immunodominant in patients with MS232,233,240. However, a plethora of antigens, such as GDP l-fucose synthase and RAS guanyl-releasing protein 2, may also have a decisive role18. Consistent with this, patients with MS demonstrate high T cell receptor (TCR) repertoire diversity and interindividual variability240. With increasing disease duration, epitope spreading further complicates AST strategies245,246. To circumvent epitope spreading, autoantigen cocktails or coupling of peptides to cells have been proposed as potential mitigation strategies247,248. The multitude of administration routes (oral, nasal, transdermal, intramuscular, intravenous, coupled to cells, or coupled to nanoparticles) and modalities (whole protein, peptides, DNA, T cell or TCR vaccinations, and tolerogenic dendritic cells) further increase the complexity of AST249. Moreover, it is also difficult to assess treatment efficacy owing to the rarity of autoreactive T cells and a lack of specific markers for them. Despite success in animal models, these challenges and other limitations, such as insufficient patient stratification, have led to many discouraging results in clinical tolerization trials250-253. We note that attempted tolerization with an altered peptide ligand of MBP83-99 has induced MS disease activity250. The use of autoantigen cocktails provided the first promising results as transdermal administration of three myelin peptides (MBP83-99, MOG35-55, and PLP139-151) reduced clinical and MRI disease activity in patients with RRMS254. However, this trial included only 30 patients and thus larger studies are required.

Nevertheless, recent advances in AST have created enthusiasm in the field. The advent of mRNA vaccines during the COVID-19 pandemic has generated great interest in exploiting this vaccination strategy to treat other conditions. Krienke et al. vaccinated mice with EAE with a nanoparticle-formulated 1-methylpseudouridine-modified mRNA (m1ψ-mRNA) that codes for multiple disease-related autoantigens (such as MOG35-55 and PLP139-151) prevented an innate immune response to the mRNA. M1ψ-mRNA vaccination resulted in autoantigen presentation by APCs without inducing costimulatory signals or cytokines (such as CD86 and IFN), which reduced the number of effector T cells, increased the number of exhausted T cells, and induced Treg cells with strong bystander immunosuppression capacity. Furthermore, the vaccine either abrogated disease development or stopped disease progression in the MOG35-55 EAE model, and controlled relapses in PLP139-151 EAE. Non-MOG antigen-specific immune responses were not affected, indicating that other functions of the immune system are not compromised. M1ψ-mRNA coding for autoantigens suppressed EAE induced by other autoantigens almost as effectively as if both antigens were identical. The observed strong bystander immunosuppression by Treg cells might therefore — at least in part — be able to compensate for epitope spreading, interindividual antigen variability and polyclonality of autoimmunity in MS. Moreover, repeat administration of M1ψ-mRNA was not compromised by induction of autoantigen-specific
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Antigen-specific tolerization strategies may also be valuable therapeutic options in MS. Their main advantages are their potential broad applicability and efficacy in several autoimmune diseases, in a range of patient cohorts. In contrast to AST, these unspecific approaches do not affect the underlying cause of the dysregulation and thus have to be administered repeatedly. Moreover, the unspecific effects might affect immune system function.

Antigen-unspecific tolerization strategies might include modulating immune checkpoints, inhibitors of which have revolutionized cancer therapy. Immune checkpoints control immune homeostasis by shifting T cell cytokine profiles from pro-inflammatory to anti-inflammatory, and by promoting and maintaining regulatory T cells. For some of these pathways, activators have been developed and tested in autoimmunity.

The fusion protein containing cytotoxic T lymphocyte antigen 4 (CTLA4) fused to immunoglobulin, abatacept, inhibits T cell activation and is approved for the treatment of rheumatoid arthritis; however, a pilot study including 20 patients with RRMS was prematurely terminated because of increased disease activity in the low-dose abatacept group. Interestingly, unblinding revealed a higher relapse rate in this group prior to investigational treatment; the negative outcome could therefore be related to a randomization error. Other co-inhibitory receptors such as T cell immunoglobulin mucin receptor 3 (TIM3, also known as HAVCR2) and programmed cell death 1 (PD1) and its ligand PD1L are potential targets in MS therapy. Dysfunction of these pathways plays a part in the pathogenesis of EAE and MS, and activation of these pathways downregulates T cell responses. Conversely, PD1/PDL1 blockade may result in hyperactivation of T and B cells, and this has been invoked as the mechanism underlying the demyelinating adverse effects of this class of cancer drugs.

Additional, unspecific tolerization strategies exploit the tolerance-inducing abilities of parasites. Patients with MS who were infected with *Ascaris lumbricoides* demonstrated low MS disease activity, and treatment with *Trichuris suis* reduced the number of gadolinium-enhancing lesions. However, the studies had considerable limitations — small sample size, no control group, unknown mechanism of action, and adverse effects. Identifying the tolerance-inducing mechanisms used by those parasites may lead to new therapeutic strategies that do not require parasite administration. The ligand-activated transcription factor aryl hydrocarbon receptor (AHR) integrates signals from environmental stressors to produce immune responses. AHR activation induces functional regulatory T cells and various other immune pathways. Activation of AHR suppresses the development of EAE and patients with MS show lower levels of circulating AHR ligands compared to healthy controls, indicating an important role in MS pathology. Therefore, AHR-activating ligands may also be used as an MS therapy. Among other effects, laquinimod activates AHR and demonstrated beneficial effects in initial clinical trials in MS. In a phase III study (CONCERTO), laquinimod protected nervous tissue (as measured by brain volume), but this treatment was not able to reduce the risk of progressive disability. Interestingly, the combination of AHR agonists and MOG<sub>13,14</sub> in nanoliposomes induced antigen-specific tolerance and strong bystander immunosuppression, which abrogated EAE. These data suggest a strategy combining AST with nonspecific tolerance induction. Furthermore, dietary intake affects the production of gut microbiome metabolites, such as short-chain fatty acids, that act as tolerance-inducing signals.

Another possible way to restore peripheral tolerance is to administer tolerogenic cells. These strategies mainly utilize regulatory T cells, but can use other cell types such as myeloid-derived suppressor cells. Regulatory T cells engineered with high-affinity TCRs or a chimeric antigen receptor also allow for antigen-specific approaches.

However, many challenges, such as stability, functional activity, cost and delivery are yet to be overcome to allow cell-based strategies in clinical practice. Nevertheless, a phase Ib/IIa clinical trial using autologous regulatory T cells in patients with MS has been completed with good safety outcomes.

Recently, the potassium channel *K<sub>Ca</sub>1.1* has been identified as a critical regulator of thymic regulatory T cell differentiation. Loss of *K<sub>Ca</sub>1.1* function reduced regulatory T cell numbers and worsened EAE. Furthermore, patients with MS who had a dominant-negative missense *K<sub>Ca</sub>1.1* variant had lower regulatory T cell numbers and worse clinical outcomes compared to non-carriers. Interestingly, pharmacologic activation of *K<sub>Ca</sub>1.1* rapidly and reversibly increased regulatory T cell numbers in humans, thus presenting a new potential therapeutic strategy to exploit tolerance induction by regulatory T cells for the treatment of autoimmune disorders.

Additional targets for antigen-specific and antigen-unspecific tolerization strategies are found in the gut-associated lymphoid tissue and the gut microbiome. Microbiota–immune system interactions are essential for immune homeostasis and imbalances are involved in a multitude of immune-mediated disorders.
The gut microbiome has been implicated in providing a pro-inflammatory environment that allows the emergence of activated myelin-specific T cells through bystander activation\(^{197}\). Through the release of metabolic products such as tryptophan derivatives that engage AHR, bacteria can regulate the cytokine milieu in the CNS and the function of neurons and glial cells\(^{174,282}\). Patients with MS have evidence of gut dysbiosis but studies have yielded heterogeneous results regarding overrepresentation and underrepresentation of bacterial species\(^{150,288}\). Frequently, the concentration of the bacterial short-chain fatty acids butyrate and propionic acid are reduced\(^{155,163}\). Supplementation with propionate, AHR ligands and orally delivered antigens can induce T<sub>reg</sub> cells via gut dendritic cells that are specifically conditioned by gut epithelial cells and microbiota\(^{199,280}\). Probiotics, prebiotics, specific diets, microbiota supplementation and faecal transplantation have been tested in clinical trials to evaluate their effects on the gut microbiome and MS disease outcomes\(^{261–263}\).

Thus, immune tolerance strategies offer promising opportunities in MS treatment. However, several challenges remain, including epitope spreading, interindividual antigen variability and polyclonality as well as infrastructural and cost limitations for cell-based therapies. Those pitfalls need to be addressed prior to their introduction to clinical practice.

Conclusions and perspectives

Remarkable progress has been made in our understanding of MS. This knowledge should facilitate the development of therapeutics that focus on the prevention of active lesion formation and early neuroinflammation. However, clinical progression, characterized by increased physical disability and cognitive impairment over time, places a great burden on affected patients. Because the long-term prevention of neurodegeneration remains a major therapeutic challenge, focusing on the mechanisms underlying neurodegeneration in MS is important. Moreover, a deeper understanding of the processes leading to neuronal and axonal degeneration would benefit the treatment of MS, but also of other diseases associated with neurodegeneration, such as Alzheimer disease or Parkinson disease.

Studying aberrations of cellular metabolism is a promising and evolving novel approach in MS. Modulating or fuelling different metabolic pathways could lead to new therapeutics. Moreover, metabolism is linked to environmental cues (such as exposure to pollutants or nutrition) and changes in the redox equilibrium in both the immune and the nervous system. However, most metabolic alterations have instantaneous effects, and metabolism can adapt if needed. This is particularly relevant in patients with co-morbidities such as diabetes or arterial hypertension, which frequently occur in the elderly and interact with MS-specific damage pathways and normal ageing processes.

Currently approved DMTs for MS focus mainly on the inflammatory aspects of the disease and target immune cell populations. However, glial cells such as oligodendrocytes maintain brain homeostasis by supporting neuronal health and contributing to endogenous regenerative processes. Therefore, new therapies improving the function of oligodendrocytes and other glial cells may aid in preventing neurodegeneration and reversing structural damage.

Several coagulation factors may prove to be suitable therapeutic targets in MS, and there are already clinically approved and well characterized drugs available for long-term use. Nevertheless, more research is needed to evaluate the potential therapeutic utility of these drugs in MS and to weigh that utility against the known detrimental side effects, such as bleeding.

Preserving the integrity of the BBB could prevent immune cell infiltration into the CNS, and ECs have a unique position next to the bloodstream, which makes them potential therapeutic targets. However, BBB dysfunction may be a prerequisite for, but not the sole cause of, MS. Therefore, inhibiting BBB breakdown could have beneficial effects but may not prevent all aspects of the disease.

Inducing immune tolerance might address the underlying causes of MS. However, many challenges remain prior to their reaching clinical practice.

Another important factor to be considered when developing CNS therapeutics is the challenge of drug delivery. New pharmacological technologies are required to precisely target specific components within the CNS tissue and must be able to cross the BBB without altering its protective function.

In conclusion, innovative MS therapies may combine strategies of promoting immunomodulation, fostering remyelination and providing neuroprotection, and future trials should pursue such a multifaceted approach to improve the long-term prognosis for this crippling disease.

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