Immunopathology of multiple sclerosis

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Abstract | Two decades of clinical experience with immunomodulatory treatments for multiple sclerosis point to distinct immunological pathways that drive disease relapses and progression. In light of this, we discuss our current understanding of multiple sclerosis immunopathology, evaluate long-standing hypotheses regarding the role of the immune system in the disease and delineate key questions that are still unanswered. Recent and anticipated advances in the field of immunology, and the increasing recognition of inflammation as an important component of neurodegeneration, are shaping our conceptualization of disease pathophysiology, and we explore the potential implications for improved healthcare provision to patients in the future.

Demyelination
Damage to the myelin sheath surrounding nerves in the brain and spinal cord, which affects the function of the nerves involved. Demyelination occurs in multiple sclerosis and in experimental autoimmune encephalomyelitis, which is an animal model of multiple sclerosis.

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Approximately 2.5 million people worldwide are afflicted with multiple sclerosis, a chronic neuroinflammatory disease of the brain and spinal cord that is a common cause of serious physical disability in young adults[1], especially women. Multiple sclerosis poses a major personal and socioeconomic burden: the average age of disease onset is 30 years — a time that is decisive for work and family planning — and 25 years after diagnosis, approximately 50% of patients require permanent use of a wheelchair. The condition has a heterogeneous presentation (BOX 1) that can include sensory and visual disturbances, motor impairments, fatigue, pain and cognitive deficits[1]. The variation in clinical manifestations correlates with the spatiotemporal dissemination of lesional sites of pathology within the central nervous system (CNS)[2]. These lesions are a hallmark of multiple sclerosis (BOX 2) and are caused by immune cell infiltration across the blood–brain barrier (BBB) that promotes inflammation, demyelination, gliosis and neuroaxonal degeneration, leading to disruption of neuronal signalling[3]. T cells appear early in lesion formation, and the disease is considered to be autoimmune, initiated by autoreactive lymphocytes that mount aberrant responses against CNS autoantigens, the precise nature of which, however, remains enigmatic.

Infiltration of immune cells from the periphery — which is particularly prominent in the common, relapsing-remitting form of the disease — has been the main target of currently available therapies for multiple sclerosis (see Supplementary information S1 (table)). Although these broad-spectrum immunomodulatory drugs reduce immune cell activity and entry into the CNS and decrease relapse frequency, they are often associated with side effects. These range from flu-like symptoms and the development of other autoimmune disorders to malignancies and even fatal opportunistic infections such as progressive multifocal leukoencephalopathy[4] (see Supplementary information S1 (table)), indicating the need to identify more specific therapeutic targets that can be efficaciously modulated but without inducing such significant adverse reactions.

Concomitantly, it has been increasingly acknowledged that although the long-standing treatments approved for multiple sclerosis can reduce relapses, they do not substantially halt the disease[4,5], and neuroaxonal damage — with ensuing physical disability — continues to accumulate and become permanent. This supports the concept that there is some degree of discord between the processes driving overt relapses and those driving chronic progression. Indeed, secondary progressive disease may not be a temporally distinct phase of the condition arising as a direct consequence of the relapsing-remitting disease but may instead be the outcome of other underlying pathophysiological mechanisms. This is also in keeping with the existence of the relapse-free, primary progressive form of multiple sclerosis (BOX 1).

The inferred uncoupling of relapses and disability progression has considerable ramifications for our understanding of disease pathways and for therapeutic design, as there are currently no drugs approved to specifically treat primary or secondary progressive multiple sclerosis[5]. Although disease progression is not greatly influenced by the available immunomodulatory therapies, which target peripheral immune cell activation and entry into the CNS, immunological involvement is implicated in this process: there is an additional inflammatory component residing in the CNS that is only marginally influenced by peripheral immune

Gliosis
The proliferation and activation of glial cells (microglia, oligodendrocytes and astrocytes) in response to damage in the central nervous system.

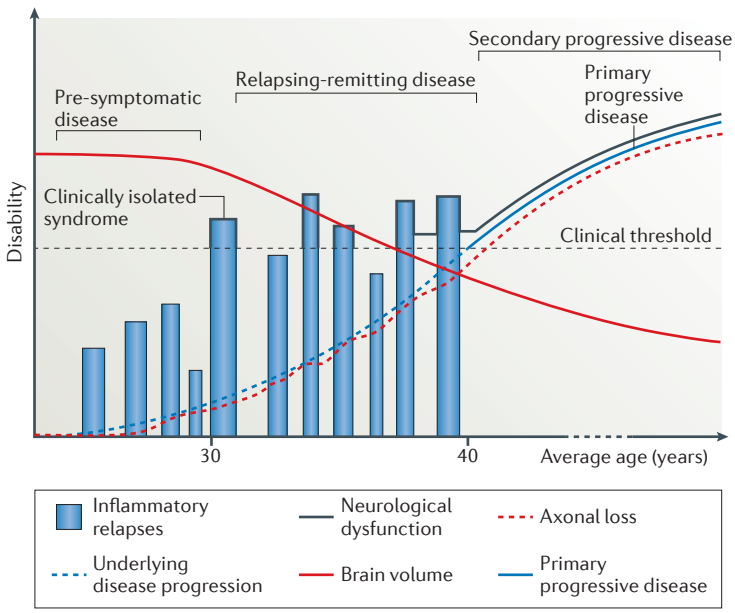
control and that contributes to gradual neuroaxonal loss and demise of myelin-producing oligodendrocytes[6–8]. This CNS-resident inflammatory arm of the disease is less well defined but is likely to involve continuous activation of innate immune cells; these cells have been found to predominate in demyelinated areas, but they are also present diffusely throughout normal-appearing white matter, and their numbers correlate with tissue damage[9].

Box 1 | The heterogeneity of multiple sclerosis

The disease course and symptomatology of multiple sclerosis are heterogeneous, although efforts to categorize patients by general patterns of disease presentation have allowed several disease subtypes to be recognized. The most common form, affecting approximately 85% of patients, is relapsing-remitting multiple sclerosis. It is characterized by an initial episode of neurological dysfunction (clinically isolated syndrome), followed by a remission period of clinical recovery and then recurring bouts of relapse and remission (see the figure; black line). Relapses coincide with focal central nervous system (CNS) inflammation and demyelination that are typically discernible, using magnetic resonance imaging, as white matter lesions. Eventually, improvement during each remission wanes as disability accumulates, and approximately 80% of patients go on to develop secondary progressive multiple sclerosis, one to two decades post diagnosis. In secondary progressive disease, inflammatory lesions are no longer characteristic, and progressive neurological decline is instead accompanied by CNS atrophy; that is, decreased brain volume and increased axonal loss (see the figure; red solid and dashed lines, respectively). Approximately 10% of patients with multiple sclerosis are diagnosed with primary progressive disease, which features progressive decline from the outset and an absence of relapses (see the figure; blue line).

No significant geographical differences have been reported in the relative prevalence of the three main disease subtypes[1]. In Asian populations, however, a different form of relapsing-remitting disease — known as opticospinal multiple sclerosis — is thought to be common, although it is debated whether this disease should in fact be classified as a neuromyelitis optica spectrum disorder rather than a form of multiple sclerosis[112]. Rarer variations of multiple sclerosis have also been reported[1], such as progressive-relapsing and paediatric disease, as well as tumefactive multiple sclerosis, which includes the particularly severe Marburg variant.

As the characterization of multiple sclerosis heterogeneity improves, a key challenge is to determine whether or not differing disease presentations can truly be classified as a single disease, and what implications diagnostic accuracy will have on the understanding of shared and distinct pathophysiological mechanisms and on therapeutic targeting.



Dissecting the distinct roles of the immune system in the events that trigger multiple sclerosis development and those that contribute to disease progression is thus complicated by the multicellular pathophysiology associated with infiltrating adaptive and innate immune cells, as well as CNS-resident innate immune cells with inflammatory capacity and by the chronic nature of the disease that unfolds over a period of many decades.

In this Review, we evaluate how our understanding of the involvement of the immune system in driving the development of multiple sclerosis is being shaped by the ongoing interrogation of genetic predisposition and environmental influences. We discuss the changing role of peripheral immune cells — including effector and regulatory lymphocytes and innate immune cells — in promoting pathogenesis as the disease takes its course, and we point to CNS-resident innate cells as emerging key contributors to chronic inflammation. In considering our current view of multiple sclerosis immunopathology, we highlight the outstanding clinical needs and the imminent biomedical challenges for the future.

What causes multiple sclerosis?

The exact cause of multiple sclerosis, and whether this varies from one patient to the next, still remains elusive, but the disease is thought to arise in genetically susceptible individuals, with stochastic events and environmental factors influencing disease penetrance. Genetic variation accounts for approximately 30% of the overall disease risk, and with the advent of genome-wide association studies (GWASs), more than 100 distinct genetic regions have been identified as being associated with multiple sclerosis, collectively explaining approximately one-third of the genetic component of the condition[10]. Despite the fact that non-genetic factors have a proportionately larger contribution than genetic factors to immunological heterogeneity in general[11], comparatively less progress has been made in elucidating environmental determinants of multiple sclerosis, perhaps reflecting the difficulty of accurately interpreting complex, and sometimes confounding, epidemiological data[12].

Without a known predominant exogenous risk factor, it is an open question whether multiple sclerosis is triggered in the periphery or in the CNS. In the CNS-extrinsic (peripheral) model, autoreactive T cells that are activated at peripheral sites — potentially through molecular mimicry[13–15], bystander activation or the co-expression of T cell receptors (TCRs) with different specificities[16] — traffic to the CNS along with activated B cells and monocytes (FIG. 1). This model is consistent with the method used to induce the multiple sclerosis-like disease experimental autoimmune encephalomyelitis (EAE) in animals: emulsified CNS antigen is administered along with immune stimulants, resulting in the generation of pathogenic $CD4^+$ T helper 1 (T_H1) cells and T_H17 cells in the draining lymph nodes. These cells then enter the circulation and ultimately exert their effector functions within the CNS, having crossed the BBB or the blood–cerebrospinal fluid (CSF) barrier at the choroid plexus (BOX 3).

Tumefactive multiple sclerosis
A subtype of multiple sclerosis characterized by atypical, large demyelinated lesions that appear tumour-like and oedematous and can exert pressure on the surrounding central nervous system tissue due to their size.

Molecular mimicry
A mechanism by which a peptide from a foreign antigen that is presented to a T cell closely resembles part of a self-protein, thereby triggering an autoimmune reaction.

Choroid plexus
The site of production of cerebrospinal fluid in the adult brain. It is formed by invagination of ependymal cells into the ventricles, which become highly vascularized.

Primary neurodegeneration
The process of progressive dysfunction and loss of axons and neurons, triggered by mechanisms involving central nervous system-resident cells, as opposed to cells infiltrating from the periphery.

Candidate genes
Genes assumed to be affected by disease-associated genetic polymorphisms, based on their functional relevance and/or their physical proximity to the polymorphisms in question. The determination of whether the assigned candidates are truly affected by the polymorphisms and how they influence disease susceptibility typically requires functional follow-up investigations at the molecular, cellular and systemic levels.

Alternatively, CNS-intrinsic events may trigger disease development, with the infiltration of autoreactive lymphocytes occurring as a secondary phenomenon. It is unclear what these CNS-intrinsic events might be, although postulated mechanisms include inflammatory responses to an as yet unknown CNS viral infection — a hypothesis based partly on the emerging appreciation of CNS immune surveillance[17,18] (BOX 3) — or to processes leading to primary neurodegeneration, similar to those that have been implicated in Alzheimer disease or Parkinson disease[19]. However, drawing support for either model of multiple sclerosis aetiology from other diseases warrants a closer consideration of how known multiple sclerosis risk factors compare to those for other common autoimmune and neurodegenerative conditions.

Genetic predisposition. The majority of multiple sclerosis-associated candidate genes are thought to be immunological. Consequently, the notable overlap in associated genomic regions between multiple sclerosis and other autoimmune diseases is not surprising[10] and may suggest some sharing of predisposing immunological processes, thereby supporting the peripheral model of multiple sclerosis initiation. However, in some cases this is only an apparent overlap: for instance, the same variant in the gene region encoding tumour necrosis factor receptor 1 (TNFR1) confers susceptibility to multiple sclerosis but promotes protection against ankylosing spondylitis, consistent with the opposing effects of drugs targeting the TNFR1 pathway, which exacerbate multiple sclerosis relapses but show efficacy in ankylosing spondylitis[20]. Despite this caveat, efforts

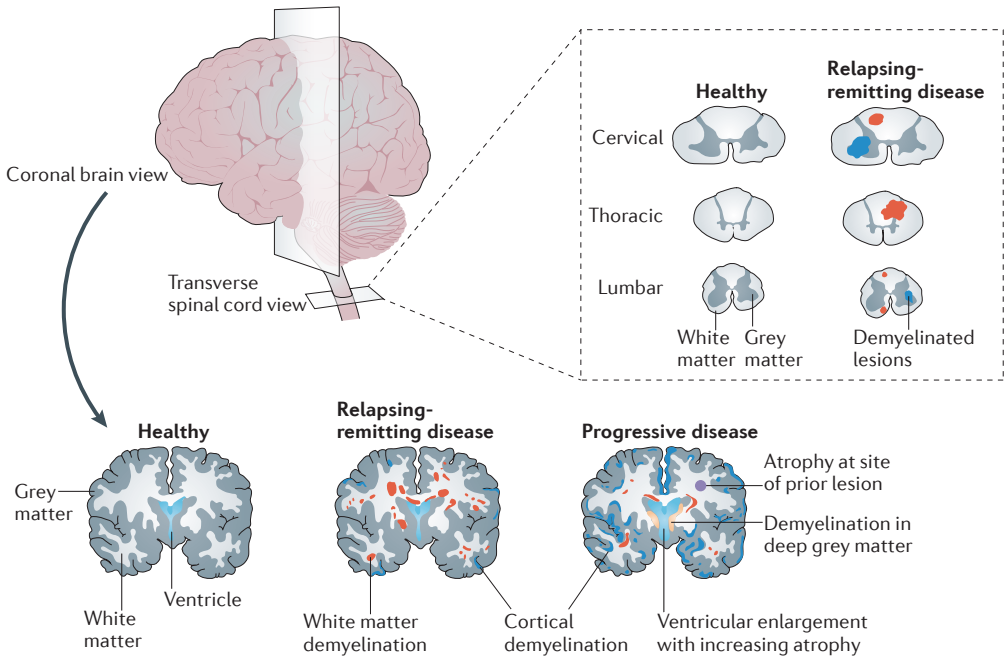
Box 2 | The pathology of multiple sclerosis

Multiple sclerosis pathology is characterized by confluent demyelinated areas in the white and grey matter of the brain and spinal cord that are called plaques or lesions and that indicate a loss of myelin sheaths and oligodendrocytes (see the figure). Although axons and neurons are mostly preserved in early multiple sclerosis, ongoing disease results in gradual neuroaxonal loss that correlates with patient disability, and the brain atrophy that occurs is accompanied by ventricular enlargement (see the coronal view in the figure). Astrocytes form multiple sclerotic glial scars in white matter lesions. Demyelinated areas in the white matter can be partially repaired by remyelination. Demyelination is also found in the grey matter of the cortex, nuclei and spinal cord[9].

Inflammation is present at all stages of multiple sclerosis, but it is more pronounced in acute phases than in chronic phases. Early lesions show invading peripheral immune cells and leakage of the blood–brain barrier. Macrophages dominate the infiltrate, followed by $CD8^+$ T cells, whereas lower numbers of $CD4^+$ T cells, B cells and plasma cells can also be found. In early disease, there is little overt damage to the brain and spinal cord in the areas outside the focal lesions, termed normal-appearing white matter, although general brain atrophy has been noted[104].

As the disease continues, diffuse inflammatory T cell and B cell infiltrates, microglia and astrocyte activation, and diffuse myelin reduction and axonal injury are evident. This results in a more pronounced atrophy of the grey and white matter[9]. Although the T cell composition of infiltrates does not differ as the disease develops, the relative proportion of B cells and plasma cells increases[3]. Microglia and macrophages remain in a chronic state of activation throughout the disease[113].

Eventually the inflammation becomes organized inside the central nervous system, with fewer invading cells observed in the lesions during progression. In secondary progressive disease, tertiary lymphoid structures have been found to form in the meninges, and these inflammatory aggregates may contribute to cortical demyelination and tissue injury at later stages[71].



to obtain a more comprehensive interpretation of the genetic data have led to the construction of interactome networks[21] using the presumed candidate genes assigned to each associated region. For multiple sclerosis, such analyses implicate the involvement of interleukin-2 (IL-2), interferons (IFNs) and nuclear factor-κB signalling, among numerous other immunological pathways, in disease predisposition[22].

These findings are consistent with pre-GWAS concepts regarding immunological mechanisms in multiple sclerosis, but the more substantial use of GWAS data to dissect disease pathways requires more in-depth investigations. Epigenetic[23,24], transcriptomic[25] and immunoprofiling[26,27] analyses are just beginning to shed light on how the variants correlate with immune cell subset-specific differences in the regulation of gene expression,

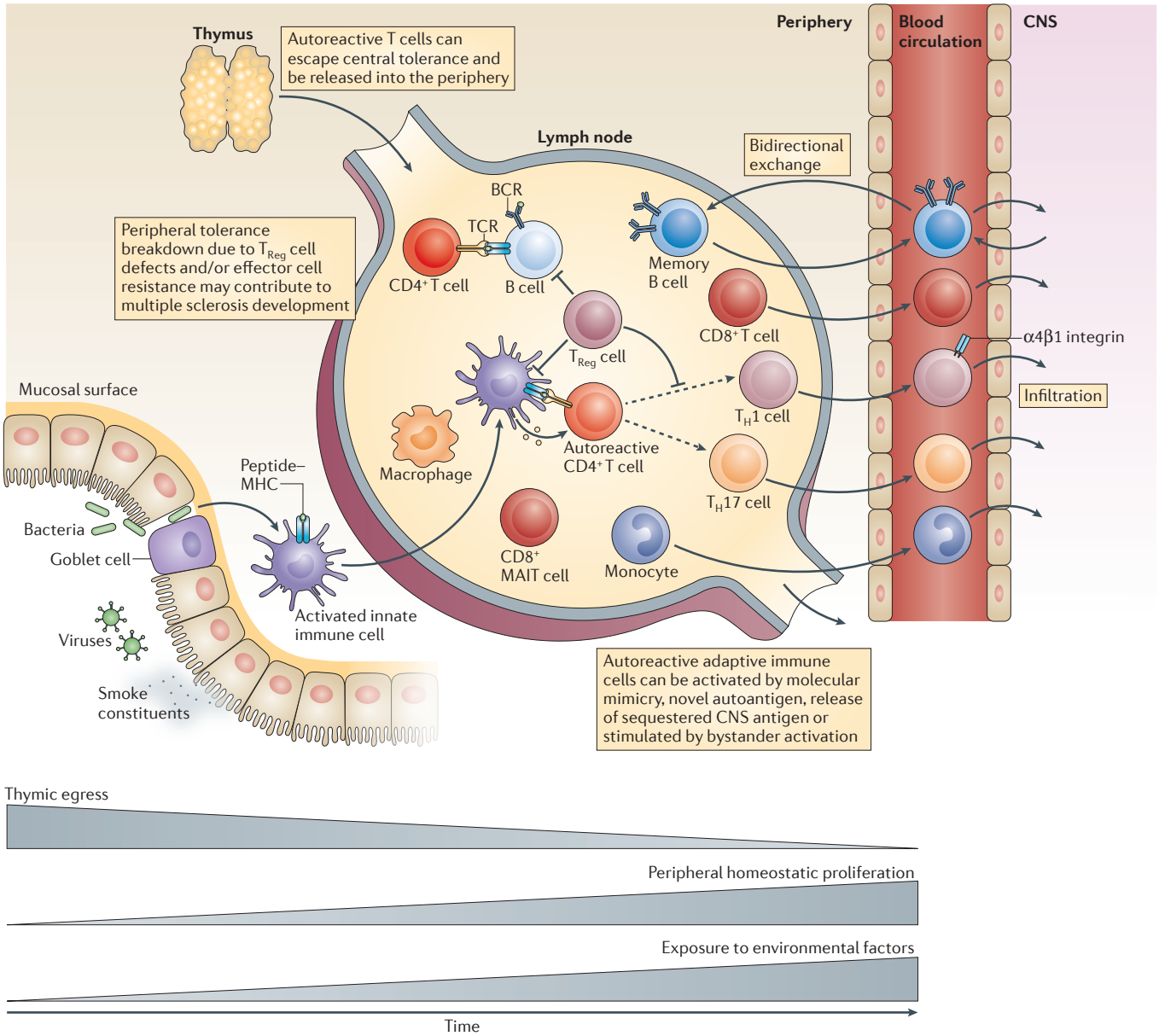


Figure 1 | **Immune system dysregulation outside the CNS.** During the establishment of central tolerance in the thymus, most autoreactive T cells are deleted; however, this process is imperfect, and some autoreactive T cells are released into the periphery. In health, peripheral tolerance mechanisms keep these cells in check. If this tolerance is broken — through the reduced function of regulatory T (T_{Reg}) cells and/or the increased resistance of effector B cells and T cells to suppressive mechanisms — central nervous system (CNS)-directed autoreactive B cells and T cells can be activated in the periphery to become aggressive effector cells by molecular mimicry, novel autoantigen presentation, recognition of sequestered CNS antigen released into the periphery or bystander activation. Genetic and environmental factors, including infectious agents and smoke constituents, contribute to these events. Once activated, $CD8^+$ T cells, differentiated $CD4^+$ T helper 1 (T_H1) and T_H17 cells, B cells and innate immune cells can infiltrate the CNS, leading to inflammation and tissue damage. B cells trafficking out of the CNS can undergo affinity maturation in the lymph nodes before re-entering the target organ and promoting further damage. Dashed arrows indicate differentiation. BCR, B cell receptor; $CD8^+$ MAIT cell, $CD8^+$ mucosa-associated invariant T cell; TCR, T cell receptor.

Interactome networks
Maps of molecular interactions, often segregated by cell type, and used as a framework to simplify cellular organization and to help address systems biology questions at the cellular level. These networks may reflect sets of physical intermolecular interactions as well as other molecules that indirectly act together in specific pathways.

Central tolerance
Self-tolerance that is created at the level of the central lymphoid organs. Developing T cells (in the thymus) and B cells (in the bone marrow) that strongly recognize self-antigen must undergo further rearrangement of antigen-receptor genes to become self-tolerant, or they face deletion.

Immune-privileged site
An area in the body with a decreased immune response to foreign antigens, including tissue grafts. These sites include the brain, eye, testis and placenta.

Dural sinuses
Venous channels located between layers of the brain dura mater. These sinuses receive blood from both internal and external brain veins, and cerebrospinal fluid from the subarachnoid space, and empty into the jugular vein.

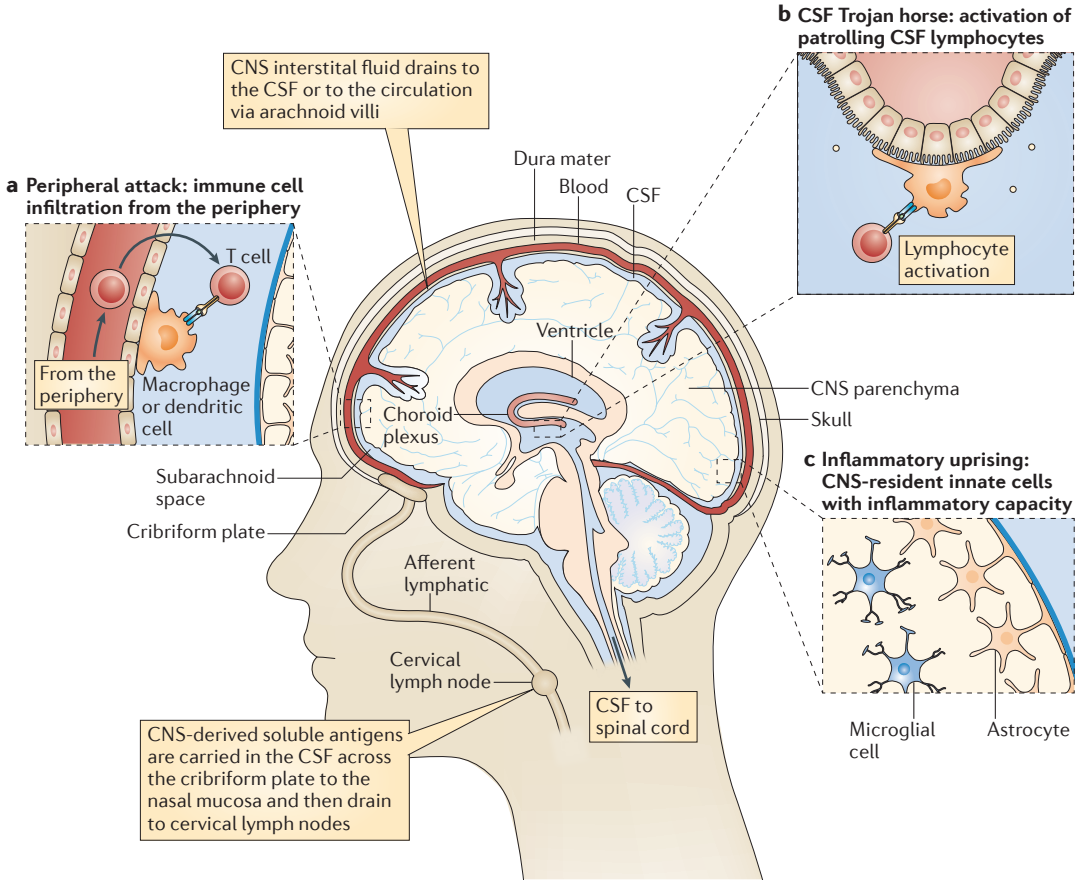
as most associated genetic variants are non-coding and many colocalize with gene enhancers or repressors in immune cells[23]. However, correlations do not necessarily reflect causality. To date, a more detailed, but not definitive, understanding of genetically determined disease pathways has been attained for only a handful of associated loci, such as the *HLA-A*02:01* and *HLA-DRB1*15:01* variants[28,29], and the genes encoding the α-chains of the IL-2 and IL-7 receptors (IL-2Rα and IL-7Rα, respectively)[30–33]. The data implicate central tolerance mechanisms, as well as peripheral differences in effector T cell function due to altered cytokine responsiveness, cytokine production and homeostatic proliferation, in multiple sclerosis predisposition.

Box 3 | **Breaching CNS barriers: peripheral attack, a CSF Trojan horse or an inflammatory uprising?**

The central nervous system (CNS) has been considered to be an immune-privileged site: administration of immunogenic agents into the CNS parenchyma typically fails to elicit an adaptive immune response. This has previously been attributed to the presence of endothelial and epithelial barriers that restrict leukocyte trafficking (including the blood–brain barrier (BBB), and the blood–cerebrospinal fluid (CSF) and blood–leptomeningeal barriers[114]), the parenchymal anti-inflammatory milieu and the anatomical isolation of the CNS from the lymphatic system. A recent study in mice, however, provides initial evidence for CNS lymphatic vessels lining the dural sinuses, suggesting that the dogma regarding the lack of anatomical connectivity between the CNS and lymphatic system may require re-evaluation[18], and warrants further investigation in humans.

The relatively immune-privileged status of the CNS has been considered as a proponent for the peripheral initiation of adaptive immune responses against CNS antigens with ensuing CNS barrier infiltration (see part **a** in the figure), but even in the healthy CNS, memory T cells traffic through the CSF, indicating a capacity for CNS-intrinsic immune surveillance[17,114]. The isolation of the CNS from the adaptive immune system is not absolute, thereby providing some potential for the initiation of an adaptive immune response in the CNS in the absence of a BBB breach. Consistent with CNS immune surveillance, blood-derived innate immune cells in the meningeal, perivascular and ventricular spaces are appropriately localized to present antigen to T cells patrolling through the CSF (see part **b** in the figure), and they may activate CNS antigen-restricted T cells under highly inflammatory conditions[52].

Behind the BBB, the CNS parenchyma contains innate, yolk sac-derived microglial cells[115] (see part **c** in the figure). These tissue-resident macrophages have diverse pro-inflammatory and anti-inflammatory roles, although their primary function is not antigen presentation. Notably, microglial cells are implicated in the pathophysiological chronic, low-grade inflammation that is associated with Alzheimer disease[19], and thus they may have a similar role in multiple sclerosis.



Although still limited, the present view regarding the functional implications of multiple sclerosis-associated genetic polymorphisms is that the *HLA* variants primarily define the CNS specificity of the disease by affecting the T cell repertoire, whereas the non-*HLA* variants more broadly influence the threshold of immune cell activation, thereby ultimately altering the likelihood of a CNS-directed autoimmune response being mounted.

Overall, strikingly few genetic associations are shared between multiple sclerosis and other neurodegenerative conditions such as Alzheimer disease[34] and Parkinson disease[35]. This indicates that non-immunological, primary neurodegenerative processes are less likely to promote the initiation of multiple sclerosis, although genetic contributions to disease severity or subphenotypes of the disease may yet reveal a role for neurological genes. Intriguingly, however, disease risk associations in the *HLA* region have also been observed for the other neurodegenerative conditions[34,35], even though T cell infiltration is uncharacteristic for these diseases, and thus further investigation is needed to determine the significance of these findings.

The genetic architecture of multiple sclerosis emphasizes the prominent role of the immune system in disease predisposition. The clinical relevance of determining the specific phenotypic consequences of multiple sclerosis-associated polymorphisms has now begun to be recognized[20], and this plethora of variants can serve as a platform for interrogating human immune system diversity: to help to fine-tune our understanding of disease immunopathogenesis, to identify more targeted treatment approaches and to even uncover novel immunological pathways that can be harnessed for therapeutic benefit.

Environmental factors. In line with the perceived distinct roles of multiple sclerosis genetic risk factors in the direct triggering of autoreactivity and in the broader modulation of thresholds of immune cell activation, the environmental factors that contribute to disease development may also fall into two similar categories.

Those environmental factors more directly involved in the triggering of autoreactive T cells are often postulated to be viral or microbial in nature and mediate their effects through molecular mimicry[13–15]. Tolerance breakdown may also arise through the environmental factor-driven generation of novel autoantigens[36]. In addition to directly providing or modifying relevant antigens, environmental determinants such as CNS-tropic infectious agents may also promote the release of sequestered CNS antigens into the periphery, as has been observed in a model of Theiler's murine encephalomyelitis virus infection[14,37] (FIG. 1).

Environmental influences with a more modulatory role may indirectly alter the activation thresholds of autoreactive T cells by triggering a pro-inflammatory milieu. Intriguingly, peripheral inflammation due to infection may also have a direct influence on the CNS: locally secreted cytokines can activate afferent nerve endings, circumventricular organ and choroid plexus innate immune cells can respond to circulating pathogen-associated molecular patterns[38,39], and pro-inflammatory cytokines at high concentrations in the circulation can be transported across the BBB and can induce signalling in perivascular macrophages[40]. The outcome of this immune system-to-CNS communication seems to typically involve the pro-inflammatory activation of microglial cells. This raises the provocative question of whether, in some cases, multiple sclerosis can result indirectly from peripheral inflammation that drives microglia-dependent neurodegeneration, without the need for a CNS-directed autoreactive response to be mounted.

Considering that the numerous non-*HLA* genetic risk factors for multiple sclerosis probably affect a multitude of immunological pathways, environmental factors that influence any one of these different pathways may also contribute to disease development. Based on this parallel, there may be just as many different environmental determinants of multiple sclerosis as there are genetic risk variants. To date, the reported environmental factors implicated in multiple sclerosis variably, but not exclusively, include vitamin D, human cytomegalovirus infection[12] and circadian disruption[41]. However, smoking and Epstein–Barr virus (EBV) infection remain the best-confirmed environmental contributors[12], although it is notable that the modest impact of their individual effects on overall multiple sclerosis risk is comparable to that of any single associated genetic variant.

There is robust evidence that high levels of EBV-specific antibodies correlate with increased multiple sclerosis risk, as does a history of infectious mononucleosis[12,42]. Several mechanisms for the role of EBV infection in multiple sclerosis development have been proposed. One hypothesis is that inadequate regulation of latent EBV infection leads to viral reactivation in the CNS, resulting in EBV-transformed B cells in the meningeal and perivascular space expressing viral proteins that could activate effector T cells[43]. Furthermore, chronic viral infection can lead to an increase in the numbers of virus-specific memory T cells[44], and this increase may be accentuated in multiple sclerosis; indeed, homeostatic peripheral T cell proliferation in response to an accelerated thymic involution has been observed in patients with relapsing-remitting disease[45]. However, there is conflicting evidence regarding whether EBV RNA or protein is present in the CNS of patients with multiple sclerosis[43,46], and this hypothesis thus remains controversial. A second hypothesis suggests that EBV may instead have a more general role in immune system dysregulation, which is in keeping with the correlation of EBV infection with the risk of developing other autoimmune diseases, such as systemic lupus erythematosus[14].

As the vastness of the human virome is just beginning to be appreciated[47], our understanding of viral involvement in multiple sclerosis is still in its infancy. This is equally true for the bacterial microbiome, the genome of which is approximately 100-times larger than the human genome, and which fluctuates in composition based on environmental factors such as diet[48]. EAE studies have demonstrated that changes to the gut microbiota, for

Epitope spreading
This term is used to describe how a self-directed immune response induced by a single peptide (or epitope) could spread to include other peptides (or epitopes) not only on the same autoantigen (intramolecular spreading) but also on other self-molecules clustered in close vicinity within the target cell (intermolecular spreading).

Diapedesis
The migration of leukocytes across the endothelium, which occurs by leukocytes squeezing through the junctions between adjacent endothelial cells.

example, can alter the incidence and severity of CNS inflammation and ensuing disease[49]. However, a direct link between the microbiota and multiple sclerosis in humans has yet to be demonstrated.

Although identifying the many environmental factors that may alter multiple sclerosis risk and comprehending their mode of action poses a particularly significant challenge, the putative ease of modifying exogenous influences and human behaviour to reduce disease risk or severity is an attractive prospect for future medical intervention.

Chronic multicellular disease development

The multifactorial nature of multiple sclerosis — involving a potential deluge of different gene–environment interactions at its inception — unfolds through a complex, highly multicellular pathophysiological process that evolves throughout the duration of the disease course (FIGS 1,2).

Autoreactive T cells. The presence of T cells within CNS lesions is detectable in the early stages of multiple sclerosis[9], and the long-appreciated *HLA* associations with the disease are thought to reflect the presentation of specific CNS autoantigens to autoreactive T cells. As demyelination is a key feature of multiple sclerosis neuropathology, myelin protein-derived antigens have been hypothesized to be the main autoreactive targets. Myelin basic protein (MBP), proteolipid protein and myelin oligodendrocyte glycoprotein (MOG), for example, have been demonstrated to be recognized by circulating $CD4^+$ T cells in patients with multiple sclerosis but also in healthy individuals, and there is conflicting evidence regarding potential differences in the frequency and avidity of these cells between the two groups[50,51]. This controversy, as well as the absence of a dominant T cell autoantigen in multiple sclerosis, may be attributed to technical limitations in detecting such autoantigens, to inter-patient variation, or to epitope spreading[52,53], but unbiased combinatorial library screening methods[54] and antigen-tolerizing approaches[55] may help to further elucidate anti-myelin immune responses in the disease.

In EAE, infiltrating $CD4^+$ T cells are re-activated in the CNS by antigen-presenting cells (APCs), including $CD11c^+$ dendritic cells (DCs), with the resulting inflammatory response leading to monocyte recruitment into the CNS, as well as naive $CD4^+$ T cell activation through epitope spreading that further fuels the inflammation[52]. T_H1 cells and T_H17 cells are the main $CD4^+$ T cell subsets implicated in disease, and thus skewing of T cell differentiation away from these subsets and towards a T_H2 cell phenotype has been a prominent therapeutic concept and is considered to be a mechanism of action of the first-line, disease-modifying therapies IFNβ[56], glatiramer acetate (Copaxone; Teva and Sanofi–Aventis)[57] and dimethyl fumarate (Tecfidera; Biogen)[58].

However, the relative importance of T_H1 cells versus T_H17 cells in multiple sclerosis pathogenesis is contentious: conflicting studies variably report the predominance of one cell type over the other at initial diagnosis and during subsequent relapses and progression[59,60], and compared with controls, patient myelin-reactive peripheral $CD4^+$ T cells expressing CC-chemokine receptor 6 (CCR6) show enhanced expression of both the respective T_H1 and T_H17 cell signature cytokines IFNγ and IL-17A[61]. Furthermore, some lesional $CD4^+$ T cells have an intermediate phenotype, expressing IFNγ and IL-17A simultaneously[62]. Despite these inconsistencies, the failure of a Phase II clinical trial in patients with relapsing-remitting multiple sclerosis following the administration of ustekinumab (Stelara; Janssen)[63] — an antibody that targets the p40 subunit that is shared by the IL-12 and IL-23 cytokines, which are involved in T_H1 and T_H17 cell differentiation, respectively — was not anticipated. Suggested explanations have included a putative inability of the drug to cross the BBB and exert an effect directly in the CNS, and a diminished importance for IL-12 and/or IL-23 at later stages of disease[63]. The very premise for the ustekinumab trial, based partly on EAE studies, has also been questioned; although EAE models (see Supplementary information S2 (table)) are indispensable for studying disease mechanisms *in vivo*, interspecies immunological differences have been recognized (BOX 4), including the essential requirement for IL-23 in T_H17 cell induction in mice but not in humans[64,65]. In addition, the function of T_H17 cells seems to differ between mice and humans. T_H17 cell-mediated granulocyte–macrophage colony-stimulating factor (GM-CSF) production contributes to chronic inflammation in EAE[66], whereas T_H1 cells and other cell subsets are the primary producers of this cytokine in humans[67].

Box 4 | The promises and pitfalls of animal models of multiple sclerosis

The induction of experimental autoimmune encephalomyelitis (EAE) can be performed in a range of different species, although rodents are most common and have greatly contributed to our understanding of autoimmunity and of inflammation-induced neurodegeneration. For example, the development of multiple sclerosis drug natalizumab resulted directly from EAE experiments: natalizumab effectively blocked α4β1 integrin, which is involved in leukocyte adhesion and diapedesis at the blood–brain barrier[116].

Next-generation transgenic and genetic engineering technologies, in combination with advances in imaging, for example, are now expanding the potential utility of EAE. Thus, EAE will remain an essential tool for preclinical and mechanistic research, enabling findings from simpler *in vitro* systems — such as stem cell-based co-cultures — to be interrogated *in vivo*. However, EAE is a reductive model that needs to be used and interpreted with care. Several key aspects of the model have to be considered when translating EAE results to multiple sclerosis:

- Disease induction: harsh induction regimens using adjuvants remain a major criticism. Despite genetically engineered mice that show spontaneous disease development (see Supplementary information S2 (table)), their use has remained limited.
- Disease course: most mouse strains, including the commonly used C57BL/6 mice, show a monophasic disease course. However, other available strains can show relapsing-remitting and more progressive disease courses (see Supplementary information S2 (table)) and need to be used in concordance with the scientific question.
- Location of central nervous system inflammation: most EAE models show focused inflammation in the spinal cord, whereas multiple sclerosis is usually dominated by brain inflammation.
- Immune cell infiltrate: due to the immunization regimen, T cell responses are heavily biased towards $CD4^+$ T cells (see Supplementary information S2 (table)), whereas $CD8^+$ T cell responses dominate in multiple sclerosis.
- Interspecies immune differences: genetic and phenotypic differences in the immune systems of mice and humans have been well documented and can have implications for the relevance of EAE findings to the human disease[117].

CD8+ T cells are found in higher frequency than CD4+ T cells in the white matter and in grey matter cortical demyelinating lesions, and their numbers closely correlate with axonal damage[3]. Consistent with a key role for these cells in disease pathogenesis, myelin-specific CD8+ T cells are readily activated by epitope spreading, even in a CD4+ T cell-driven EAE model, with this being aided by antigen cross-presentation by monocyte-derived DCs in the CNS[53]. Non-myelin astrocyte-derived antigen can also trigger spontaneous relapsing-remitting disease in mice by driving the establishment of non-recirculating resident memory-like CD8+ T cells within the CNS[68]. The disease course and pathology in this model was modulated by B cells and by viral triggering, suggesting that complex multicellular and environmental interactions can contribute to disease heterogeneity. In humans, up to a quarter of CD8+ T cells in the active lesions of patients with multiple sclerosis can produce IL-17 and are thus thought to be mucosa-associated invariant T cells (MAIT cells)[69]. Efficacious autologous haematopoietic stem cell transplantation in patients with highly active disease results in the long-lasting depletion of these cells, suggesting that they have an important role in disease pathogenesis[70]. The precise contribution of CD8+ T cells, compared with that of CD4+ T cells and other cell types, following autologous haematopoietic stem cell transplantation and the relative importance of targeting these cells in the therapeutic efficacy of broad-spectrum drugs — such as natalizumab (Tysabri; Biogen and Elan), alemtuzumab (Campath-1H; Genzyme) or fingolimod (FTY720 and Gilenya; Novartis)[4] — is not entirely clear and requires further study; however, based on evidence published to date, the exploration of CD8+ T cell-specific therapies in the future is warranted.

Autoreactive B cells. Compared with T cells, infiltrating B cell numbers in the CNS vary more throughout disease progression. Clonally expanded B cells can be found in the meninges, parenchyma and CSF, and intrathecal B cells produce antibodies that are detectable in the CSF and are of diagnostic value. Numbers of antibody-secreting plasma cells are increased with age in patients with primary or secondary progressive multiple sclerosis[3]. The meninges of patients with secondary progressive disease often contain tertiary lymphoid structures of aggregated plasma cells, B cells, T cells and follicular DCs (FDCs)[71], which are a product of long-term inflammation as observed in other chronic inflammatory or infectious diseases[72]. By contrast, primary progressive disease is characterized by diffuse meningeal infiltration without such structures[73]. Despite initial reports that certain autoantigens — including MOG, neurofascin, contactin and the ATP-dependent inwardly rectifying potassium channel KIR4.1 — are recognized by pathogenic B cells in subgroups of patients, these findings still await verification[74]. Moreover, other antibody-mediated neurological diseases, such as myasthenia gravis, neuromyelitis optica and autoimmune encephalitis, show a clinical uniformity[75] that is absent in the subgroup of patients with antibody-positive multiple sclerosis.

In the absence of known autoantigens, the mechanisms controlling B cell activation, selection and affinity maturation have been a matter of speculation. However, the recent application of next-generation sequencing technologies to analyse B cell receptor diversity has allowed for the characterization of B cell clonotypes in the peripheral compartments and the CSF of patients with multiple sclerosis, and such studies indicate that antigen-experienced B cells can undergo maturation in draining cervical lymph nodes before transmigration to the CNS[76,77]. These data imply a therapeutic potential for the peripheral modulation of specific B cell subtypes[76,77]. Currently, Phase II clinical trials have shown that CD20-specific monoclonal antibodies rituximab (MabThera; Roche)[78] or ocrelizumab (Roche and Biogen)[79] are efficacious in reducing relapse rates. These drugs deplete the majority of B cell subsets but not autoantibody-producing terminally differentiated plasma cells, and they may thus serve to effectively reduce B cell-mediated antigen presentation and other non-autoantibody-associated pathogenic contributions such as pro-inflammatory IL-6 production[80].

Defective regulatory cells. The emergence and action of autoreactive B cells and T cells in multiple sclerosis may be due to the defective functions of regulatory cells, such as forkhead box P3 (FOXP3)-expressing CD4+ regulatory T (T_{Reg}) cells[81] and IL-10-producing T regulatory type 1 (T_R1) cells[82]. Although few such cells are present in the CNS of patients[83], disease-associated HLA class II variants could skew thymic selection such that the regulatory T cells that are released into the periphery inadequately suppress autoreactive effector T cells[81]. Alternatively, dysfunction of peripheral suppressor cells could be indirectly driven by the dysregulation of tolerogenic APCs, as shown in EAE[84]. Non-*HLA* genetic associations, such as variation in the *BACH2* gene region[10], may also be implicated in altering T_{Reg} cell function, as the transcription factor BACH2 has an essential role in the development of these cells[85] and acts as a super-enhancer for T cell identity[86]. However, patients with immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), who have a FOXP3 deficiency, do not develop CNS-directed autoimmunity[87], and therefore T_{Reg} cell dysfunction in patients with multiple sclerosis may be an acquired rather than a primary defect.

Studies have variably reported a decreased frequency and/or suppressive capacity of T_{Reg} cells[88,89], as well as an altered frequency of specific T_{Reg} cell subsets (such as CD39+ cells[90]), in the periphery of patients with multiple sclerosis compared with controls. Such defects have been attributed to reduced frequencies of naive circulating T_{Reg} cells of recent thymic origin (identified as $CD45RA^+CD31^+$), along with the compensatory but ineffective expansion of the memory T_{Reg} cell population[88]. Another possibility is the skewing of T_{Reg} cells towards an IFNγ-secreting T_H1 cell-like phenotype in patients, which is reversible upon IFNβ therapy[91]. An alternative but non-mutually exclusive explanation for the action of autoreactive effector T cells in multiple sclerosis is that, rather than being

Cross-presentation
The initiation of a CD8+ T cell response to an antigen that is not present within antigen-presenting cells (APCs). This exogenous antigen must be taken up by APCs and then re-routed to the MHC class I pathway of antigen presentation.

Mucosa-associated invariant T cells
(MAIT cells). A type of CD8+ T cell that is enriched at mucosal sites and is characterized by the expression of a semi-invariant T cell receptor (a dimer of Vα7.2 in combination with Jα12, Jα20 or Jα33) and is restricted by the non-polymorphic, highly evolutionarily conserved MHC class Ib molecule, MR1.

Tertiary lymphoid structures
Organized lymphocytic aggregates that form in sites of chronic inflammation. Typically, B cell- and T cell-rich zones are segregated, and dendritic cells (DCs), germinal centres with follicular DC networks and specialized endothelial cells are present.

Super-enhancer
A cluster of regulatory elements within a genomic region, often particularly enriched in sites that bind transcriptional co-activators.

Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
(IPEX). A disease caused by mutations in *FOXP3* (which encodes forkhead box P3) and characterized by refractory enteritis and, in some patients, autoimmune endocrinopathies, autoimmune diabetes and thyroiditis.

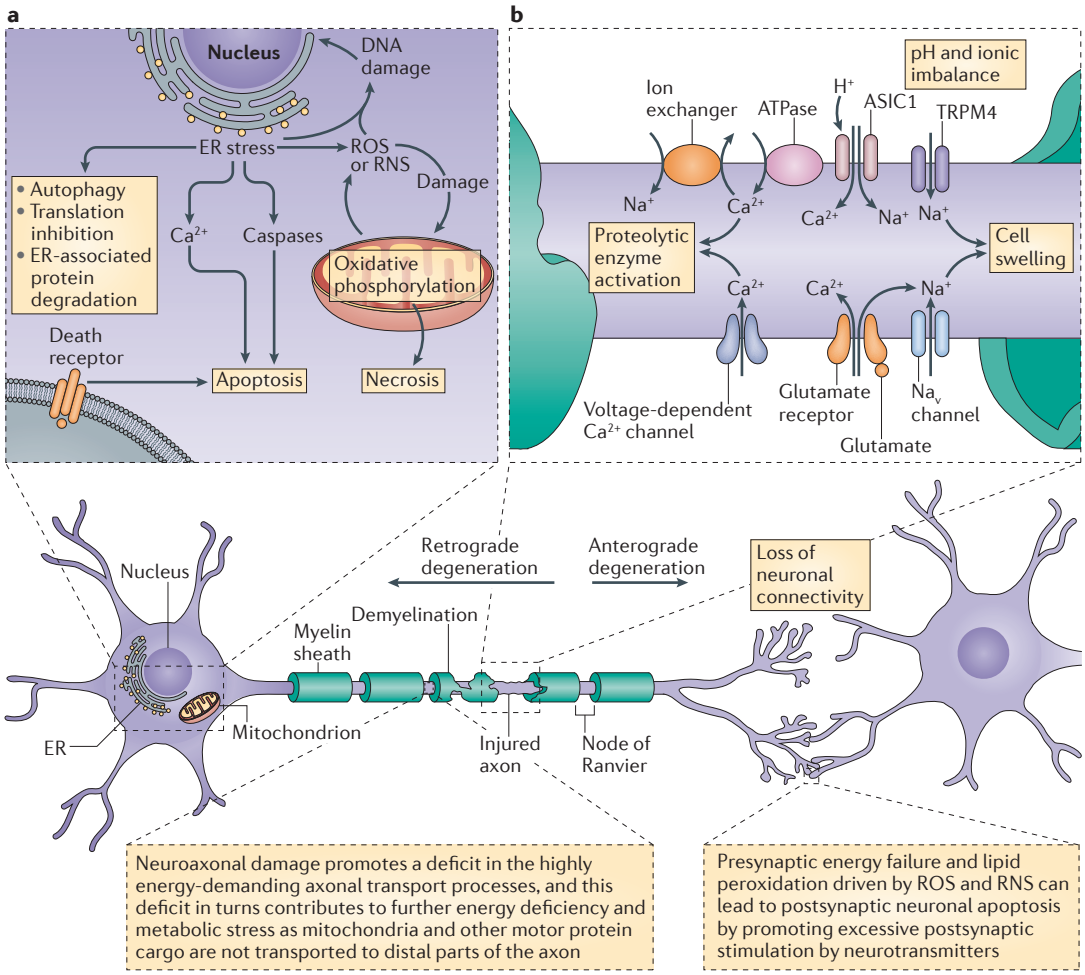
the outcome of inadequate peripheral suppression, the effector T cells themselves are actively resistant to suppressive mechanisms, with the suggestion that IL-6-induced signal transducer and activator of transcription 3 (STAT3)-mediated signalling contributes to this resistance[92,93]. Such resistance mechanisms emphasize the putative caveat of studies that document patient T_{Reg} cell dysfunction using autologous effector T cells, as these reports may in fact reflect increased effector T cell resistance.

Box 5 | **Key neurodegenerative processes as a consequence of chronic inflammation**

Chronic inflammation in multiple sclerosis results in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that probably promote mitochondrial injury as a result of the accumulation of detrimental mitochondrial DNA mutations[113,118,119] (see part **a** in the figure). This promotes metabolic stress, protein misfolding in the endoplasmic reticulum (ER), energy deficiency and a loss of neuronal fitness, which have critical implications due to the fact that transport along axons to maintain normal neuroaxonal function is highly energy demanding. Emphasizing the importance of this cascade of events in disease progression, several single-gene mitochondrial and neurometabolic disorders present as multiple sclerosis phenocopies based on certain clinical and radiographical features[120].

Several different neuronal ion channels — such as acid-sensing ion channel 1 (ASIC1), transient receptor potential cation channel subfamily M member 4 (TRPM4) and voltage-gated sodium channels ($Na_v1.2$ and $Na_v1.6$) — display a compensatory redistribution along demyelinated neurons in multiple sclerosis to help to maintain ionic homeostasis[6,7,110,121]. However, this redistribution, along with the excess accumulation of glutamate — which is the main excitatory neurotransmitter in the central nervous system and which is excessively released during neuronal injury — promotes an ionic imbalance that only serves to perpetuate tissue damage (see part **b** in the figure). From the initial site of axonal injury, these degenerative mechanisms can spread backwards towards the neuronal cell body (termed retrograde degeneration or 'neuronal dying back') or towards the distal axon terminal (termed anterograde degeneration or Wallerian degeneration) and can also influence nearby presynaptic and postsynaptic neurons, respectively, eventually leading to neuronal apoptosis or necrosis.

A range of buffering mechanisms are triggered to counterbalance neuroaxonal injury, such as an upregulation of the expression of pro-survival genes and the action of the cannabinoid system, but eventually the increasingly destructive inflammatory milieu overrides them and fundamental neuroaxonal damage follows[6]. Therefore, although there has been an interest in therapeutically boosting neuroprotective pathways as well as remyelination in multiple sclerosis, such putative therapies would probably be most efficacious when administered along with anti-inflammatory agents.



In addition to $CD4^+$ T_{Reg} cells, $CD8^+$ regulatory T cells have been implicated in EAE[94]. These cells have also been found in patients with multiple sclerosis in whom HLA-E-restricted $CD8^+$ T cells display a less regulatory phenotype than those in healthy individuals[95], and neuroantigen-specific $CD8^+$ T cells may have less suppressive capacity during relapses[96]. Enhanced cytotoxic $CD8^+$ regulatory T cell function has also been observed in patients following glatiramer acetate therapy[97], and expansion of a putative regulatory $CD103^+CD8^+$ T cell subset has been reported in some patients treated with natalizumab[98]. Drug administration may also influence regulatory B cells: IFNβ therapy correlates with an increase in the numbers of IL-10-producing regulatory $CD19^+CD24^{hi}CD38^{hi}$ transitional B cells in treated patients with multiple sclerosis[99]. Other regulatory B cell subsets, such as those secreting IL-35, have also been implicated in recovery from EAE[100].

Collectively, dysregulation of effector–regulatory cell interactions occurs in multiple sclerosis, ultimately resulting in the emergence of autoreactive adaptive immune cells that are capable of infiltrating and promoting damage within the CNS. The skewing of effector–regulatory cell interactions may provide some therapeutic benefit but may not be sufficient to prevent neurodegeneration.

Inflammation in progressive neurodegeneration

As currently available immunomodulatory therapies decrease relapse rates but not necessarily long-term multiple sclerosis progression, it has been suggested that autoimmune response-instigated neuroaxonal injury triggers a potentially self-sustaining chronic neurodegenerative process. This proceeds even in the absence of continued immune cell infiltration from the periphery, which eventually wanes regardless of therapy, possibly due to immune cell exhaustion associated with chronic antigenic exposure[101]. Although neurodegeneration in multiple sclerosis is thought to be the culmination of a cascade of events occurring in axons and neurons — including oxidative stress responses, energy deficiencies, ionic imbalances, and the failure of neuroprotective and regenerative mechanisms[6] (BOX 5) — chronic CNS inflammation may fuel these processes through the action of cells that have become or are already resident within the CNS (FIG. 2).

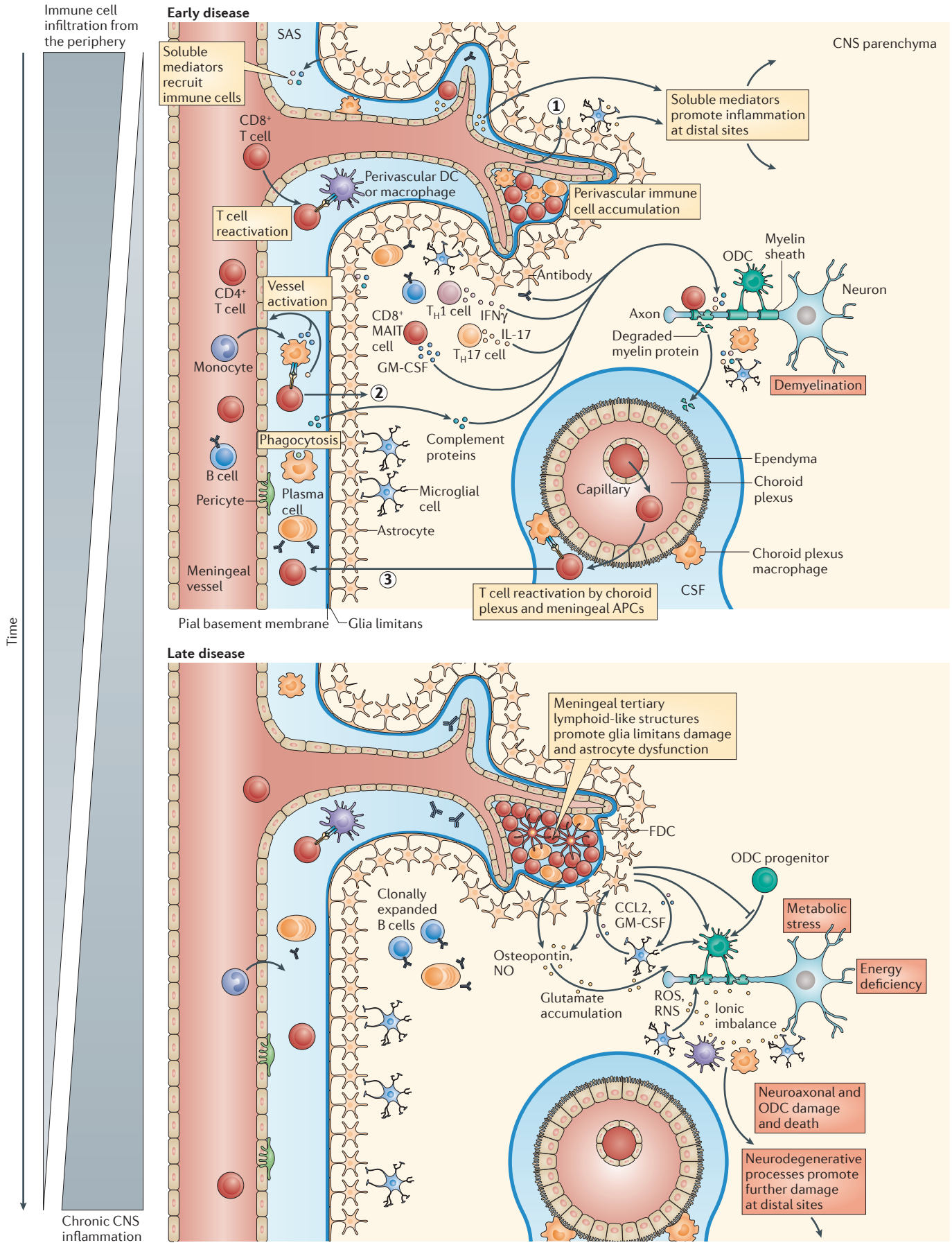
Previously infiltrating adaptive immune cells may contribute to long-term inflammation in multiple sclerosis through the eventual establishment of tertiary lymphoid structures within the CNS[71]. However, it is becoming increasingly apparent that CNS-resident cells that sense homeostatic disturbances, mainly microglia and astrocytes, can also produce a range of neurotoxic inflammatory mediators (such as cytokines, chemokines and reactive oxygen species) that promote and sustain neuroaxonal damage and thus neurodegeneration[6,19] (FIG. 2).

Moreover, these cells are likely to have a role in multiple sclerosis-associated CNS inflammation not only during the later stages of the disease when immune cell infiltration from the periphery subsides but also from the outset (FIG. 2). Even after the very first manifestation of disease, increases in the numbers and activation status of microglia and macrophages can be observed in lesions and in the normal-appearing white matter[9,102]. In addition, as neuroaxonal degeneration disseminates (BOX 5), microglia in the vicinity of axons emanating from distally damaged neurons may become activated; these cells may hence serve as the nucleus of new lesion formation[103] and might also contribute to the general brain atrophy that is observed in early disease[104]. Notably, the relative role of microglia versus monocyte-derived macrophages throughout the course of multiple sclerosis has not been fully elucidated owing to the difficulty in distinguishing these two morphologically and functionally similar cell types.

Some insights have been gained from transgenic EAE models, which have enabled these cell types to be studied in distinction: these studies have shown that at disease onset monocyte-derived macrophages initiate demyelination, whereas microglia may be more involved in debris clearing[105]. This suggests that microglia may have some neuroprotective capacity by helping to resolve inflammation[105], as well as by actively displacing specific neuronal synapses to maintain CNS homeostasis[106] and by producing neurotrophic factors (such as brain-derived neurotrophic factor) to aid the repair of

Figure 2 | **Immune system dysregulation inside the CNS in early and late multiple sclerosis.** Immune cell infiltration from the periphery is a prominent feature of early-stage multiple sclerosis (top panel) and can occur from the meningeal blood vessels by direct crossing of the blood–brain barrier (denoted '1' in the figure) or the subarachnoid space (SAS; denoted '2'), or from the choroid plexus across the blood–cerebrospinal fluid (CSF) barrier (denoted '3'). Peripheral innate and adaptive immune cells can accumulate in perivascular spaces and enter the central nervous system (CNS) parenchyma. These cells, along with activated CNS-resident microglia and astrocytes, promote demyelination and oligodendrocyte (ODC) and neuroaxonal injury through direct cell contact-dependent mechanisms and through the action of soluble inflammatory and neurotoxic mediators. Later on in the disease (bottom panel), immune cell infiltration wanes, perhaps due to adaptive immune cell exhaustion from chronic antigen exposure. However, chronic CNS-intrinsic inflammation and neurodegeneration continue. Meningeal tertiary lymphoid-like structures, which have specifically been documented in secondary progressive disease, may contribute to late-stage inflammation in patients with this form of multiple sclerosis. The action of the CNS-resident innate cells may contribute to chronic inflammation irrespective of the precise disease subtype. Stimulated by the microglia, astrocytes can produce CC-chemokine ligand 2 (CCL2) and granulocyte–macrophage colony-stimulating factor (GM-CSF), leading to even further microglial recruitment and activation, and the astrocytes can prevent remyelination at sites of neuroaxonal injury by inhibiting progenitor cells from developing into mature ODCs. APC, antigen-presenting cell; $CD8^+$ MAIT cell, $CD8^+$ mucosa-associated invariant T cell; FDC, follicular dendritic cell; IFNγ, interferon-γ; IL-17, interleukin-17; NO, nitric oxide; RNS, reactive nitrogen species; ROS, reactive oxygen species; T_H1 cell, T helper 1 cell.

Exhaustion
Non-responsiveness of the immune system resulting from the deletion of specific thymocytes (central tolerance) and the deletion or functional inactivation of specific T cells in the periphery (peripheral tolerance) in the presence of large quantities of antigen.



neuroaxonal damage. Primary neurodegeneration in conditions such as Alzheimer disease is partly attributed to inadequate neuroprotective microglial action[19], and this functional failure may also be relevant to multiple sclerosis development.

Conversely, healthy neurons constitutively express inhibitors that block the phagocytic capacity of microglia[107], implying that if left unchecked, microglia may promote tissue injury, driving a feedback loop of progressive neuroaxonal damage. Moreover, specific transgenic targeting of microglia has also been reported to reduce EAE-associated CNS inflammation[108]. In addition, activated microglia can promote astrocyte dysfunction. Similarly to microglia, astrocytes can display both pro-inflammatory and anti-inflammatory properties, and they have a crucial CNS barrier function by forming the glia limitans that lines the neuronal tissue. Their dysfunction can permit and even facilitate peripheral immune cell infiltration early in multiple sclerosis through the production of chemokines. Moreover, upon activation by stimulated microglia, astrocytes can produce CC-chemokine ligand 2 (CCL2) and GM-CSF, leading to even further microglial recruitment and activation, and they can prevent remyelination at sites of neuroaxonal injury by inhibiting the generation of mature oligodendrocytes[8] (FIG. 2). Therefore, targeting pro-inflammatory mediators produced by astrocytes may serve to inhibit both peripheral immune cell infiltration and the continuous inflammation within the CNS, and may thus be of therapeutic value.

The incompletely resolved role of CNS-resident innate-like immune cells in multiple sclerosis immunopathology — in dampening down inflammation and/or actively contributing to it — may reflect our only partial understanding of how the function of these cells varies across different regions of the CNS and throughout the course of the disease. Notably, the pro-inflammatory action of CNS-resident innate-like immune cells in progressive neurodegeneration may be intrinsically linked to multiple sclerosis chronicity. Inflammation in the CNS may be viewed as a stress response to maintain tissue homeostasis[109] — particularly as both the innate immune and neuronal compartments of the CNS are specialized to sense an array of stressors including ions, low pH[110], temperature changes, hormones and cytokines — and even in the absence of disease, CNS-derived inflammatory processes increase as a function of time, eventually promoting ageing-associated neurodegeneration[111].

In multiple sclerosis, the additional inflammation induced and/or enhanced by peripheral immune cell filtration and by CNS-resident innate-like immune cells may effectively contribute to the acceleration of the inevitable ageing processes in the CNS, and as the subjugating burden of the stress response is too great for homeostasis to be adequately maintained, pronounced progressive neurodegenerative decline follows.

Concluding remarks

Our understanding of multiple sclerosis immunopathology has been consistently modified since the approval of the first immunomodulatory therapy for the condition, and therefore questions regarding the mechanisms underscoring the triggers and long-term development of the disease remain to be definitively answered, although these questions are now better defined.

Perhaps most significantly, the appreciation of multiple sclerosis as the pathophysiological intersection between interlinked but not entirely interdependent autoimmune and neurodegenerative processes has set imminent research challenges. There is a dire need to meaningfully integrate rapidly emerging technologies and data with existing neuroimmunological clinical concepts in order to interrogate the multicellular interplay that unfolds within the CNS throughout disease progression. Underscoring this is the requirement to further decompartmentalize the study of immunology and neurology in multiple sclerosis through the investigation of neuroinflammation as a whole in order to better delineate which inflammatory and neurodegenerative mechanisms are truly distinct but occur in parallel and which are inextricably associated, so as to aid the design of more effective therapeutic strategies.

A goal for future treatment of multiple sclerosis may thus be the simultaneous, early targeting of peripheral immune cell function and of CNS-intrinsic inflammation, potentially through combinatorial therapies designed to effectively and specifically modulate these two immunological arms of the disease, along with the provision of neuroprotective or neuroregenerative drugs. Improved disease prognosis and potential patient stratification for more directed healthcare provision are also much-anticipated prospects and may become tangible as we move into the 'immune informatics' era and as large-scale, organized health resources become increasingly accessible.

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Competing interests statement
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