Multiple Sclerosis: Immunopathological heterogeneity and its implications

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Abbreviations

BBB: blood brain barrier, BCSFB : blood-cerebrospinal fluid barrier, CNS : central nervous system, EAE: experimental autoimmune encephalomyelitis, MS: multiple sclerosis,

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Abstract

Multiple sclerosis (MS) is the most common autoimmune demyelinating disease of the central nervous system (CNS). For the past decades several immunomodulatory disease modifying treatments with multiple presumed mechanisms of action have been developed, but MS remains an incurable disease. Whereas high efficacy, at least in early disease, corroborates underlying immunopathophysiology, there is profound heterogeneity in clinical presentation as well as immunophenotypes that may also vary over time. In addition, functional plasticity in the immune system as well as in the inflamed central nervous system further contributes to disease heterogeneity. In this review, we will highlight immune-pathophysiological and associated clinical heterogeneity that may have an implication for more precise immunomodulatory therapeutic strategies in MS.

Introduction

Multiple sclerosis (MS) is a demyelinating neuroimmunological disorder affecting the central nervous system (CNS). Due to MS heterogeneity, MS immunotherapy should ideally be tailored to patient phenotypes and pathomechanisms. "Precise" immunotherapy is important to achieve the optimum benefit risk profile of a therapeutic substance in the individual patient, especially in view of the limited capacity for regeneration of the CNS which determines a certain "window of opportunity", i.e. prophylactic immunotherapy before irreversible tissue damage has accrued. Thus, MS is a highly attractive candidate for strategies to stratify therapeutic interventions according to underlying disease mechanisms for specific patient groups [1]. The growing spectrum of unanticipated severe adverse drug reactions associated with different immunotherapies further underscores the need for "precise" (or "individualized, personalized") therapeutic approaches. In this review, we will discuss immunopathophysiological heterogeneity both as a major contributor and challenge to precision medicine and emphasize current approaches to stratify disease phenotypes using disease markers.

Multiple sclerosis: Clinical and pathological heterogeneity

Among patients, MS is often described as the disease of "1000 faces". This in part is owed to the multitude of symptoms that can occur in an unpredictable fashion, among others visual disturbance, motor impairment, bladder, and sexual dysfunction as well as cognitive symptoms and fatigue. Given the many potential layers of impairment and the fact that far more than two million individuals are affected worldwide often during decisive years of their professional careers and social relationships, the burden of disease and socioeconomic implications are immense [2]. Despite the presumed multifactorial etiology of MS, the initial clinical manifestation in more than 80% of patients follows a relapsing-remitting phenotype with relapses characterized by acute occurrence of new or worsening of pre-existing neurological symptoms [3]. After approximately one decade the disease phenotype often transforms into a secondary progressive disease course (SPMS) with chronic accrual of disability; a subset of patients (10-15%) progresses from disease onset (primary progressive, PPMS). The "disease of the 1000 faces" also refers to treatment response that is not readily predictable for an individual patient. With far more than a dozen immunotherapies approved there is still no uniformly valid,

evidence-based treatment algorithm that would encompass even "simple" clinical situations, i.e. choice among the different options in the first line therapy in a newly diagnosed patient. These considerations become even more complicated for sequential treatment (e.g switch due to lack of efficacy, side effects), with vulnerable patient groups (children, elderly), or a stop of immunotherapy. One major contributor to variable treatment response appears to be pathophysiological heterogeneity. The current concept stipulates that MS is a chronic inflammatory CNS disease of autoimmune etiology, influenced by genetic and environmental factors [3]. Histopathologically defined active plaques in early MS are characterized by focal white matter demyelinating lesions with perivenular cellular infiltrates consisting mainly of CD8+ T cells, CD20+ B cells and plasmablasts [4]. To some extent perivascular cuffs, but rather the associated blood-brain barrier (BBB) impairment can be detected by magnetic resonance imaging (MRI) with relatively distinct radiological appearance ("central vein sign") [5]. BBB impairment detected by MRI (i.e. lesions enhancing with the paramagnetic contrast agent gadolinium) serves as a paraclinical marker of focal "disease activity". Whereas these characteristics appear to be predominant in early relapsing MS, in progressive disease morphologically distinct, CNS-compartimentalized inflammatory reactions can be identified [4]. Here, an accumulation of T and B cells can especially be observed in meninges (spatially associated with cortical lesions) and perivascular spaces (associated with white matter lesions) with little infiltration into the lesion parenchyma and both also characterized by microglial activation and macrophage infiltration [6]. Furthermore, subpial cortical demyelination [7], diffuse white matter injury [8], slow expansion of pre-existing lesions [9, 10] and extensive brain atrophy [11] constitute histopathological features of progressive MS. In addition, albeit not visible by MRI imaging, histopathological studies on post-mortem MS brain tissues have provided evidence for continued abnormalities of BBB function in inactive MS lesions as well as in normal appearing white matter (NAWM) during progressive MS [12, 13].

Robust treatment results of immunotherapy are especially seen in early relapsing MS; in chronic progressive disease treatment response is limited which indicates that morphological heterogeneity of the inflammatory reaction may also translate into functional differences [3]. Traditionally, a two-stage process of progression has been

suggested, where an initial phase is predominantly driven by more focal inflammatory changes followed by a secondary phase where neurodegenerative and chronic inflammatory mechanisms of the disease become more dominant without evident relapses. More recently, this view of temporally distinct processes was challenged by the demonstration of insidious accrual of disability already in patients classified as early relapsing remitting MS (RRMS) [14]. In addition, in post hoc analyses of clinical trials most accumulation of disability was not associated with clinical relapses but resulted from a steady "progression independent of relapse activity (PIRA)" [15]. These data would rather argue for a continuum of focal and diffuse (compartimentalized) inflammatory and degenerative mechanisms that occur throughout the disease spectrum. However, the "PIRA" concept is mainly based on clinical outcome parameters which are prone to variability, and objective biomarkers that would be able to define or even predict these different phenotypes with presumed underlying pathomechanisms are only beginning to emerge [16].

Despite the broad range of potential pathophysiological mechanisms involved, currently approved therapies focus on relatively few presumed targets (i.e. effector T cells, B cells, immune cell trafficking into the CNS) [3, 17]. These therapies comprise substances with potentially pleiotropic mechanisms of action but also monoclonal antibodies with restricted targets [18]. The function of key inflammatory elements are determined by complex interactions, e.g. associated with the disease stage and subtypes, CNS region, or simply age-dependent aspects which all will influence treatment response.

Key inflammatory effectors, and heterogenous stage-/phase-specific roles

In the following sections we will discuss the disease specific role, functional heterogeneity and also plasticity of the targets that may govern heterogeneity of treatment response, including the brain barriers, T cells, B cells and myeloid cells.

The brain barriers

The endothelial BBB maintains CNS homeostasis by protecting the CNS from the constantly changing milieu in the blood stream. The BBB is established by brain microvascular endothelial cells, which inhibit free paracellular diffusion of water-soluble molecules by complex tight junctions [19]. Combined with an extremely low vesicular activity inhibiting transcellular passage the BBB establishes a physical permeability barrier [20]. At the same time the BBB establishes a metabolic barrier, in which polarized expression of specific transporters and enzymes ensures that toxic metabolites are rapidly removed from the CNS. These transporters may also be relevant for (heterogenous) distribution of therapeutic agents [21]. Importantly, the biochemically unique BBB characteristics are not intrinsic to brain microvascular endothelial cells but rather rely on the continuous cross-talk involving wnt/β-catenin and hedgehog signaling pathways with pericytes and astrocytes surrounding the CNS microvessels [20, 22].

The BBB also controls immune cell entry into the CNS. In the absence of neuroinflammation, the BBB limits immune cell trafficking mainly to effector and central memory T cells, which can reach the cerebrospinal fluid (CSF) compartments, but are separated from the CNS parenchyma by the glia limitans, ensuring CNS immune surveillance [23]. Under neuroinflammatory conditions additional immune cell subsets cross the altered BBB and eventually the glia limitans inducing clinical signs associated with MS [24, 25]. Migration of T cells across the BBB is a multi-step process regulated by the sequential interaction of different signaling and adhesion molecules on the BBB endothelium and the T cells [26]. Accounting for the extreme tightness of the BBB, T-cell migration across the BBB is characterized by unique adaptations. CD4 T cells crawl for long distances against the direction of blood flow on the surface of the BBB endothelium in search for rare sites permissive for paracellular diapedesis preferentially through the tricellular endothelial junctions [27-30]. During neuroinflammation the BBB endothelium presents an altered phenotype leading to the increased infiltration of presumably pathogenic CD4^{+} T cells (i.e. Th 1 or Th17 cells) and other immune cell subsets, into the CNS. Interestingly, this is accompanied by increased numbers of T cells crossing the inflamed BBB endothelium preferentially via a transcellular pathway albeit endothelial junctional integrity is impaired at this time [27, 28]. While interaction of $\alpha4\beta1$-integrins with endothelial VCAM-1 mediates T-cell arrest on the BBB under physiological flow, subsequent polarization and crawling of T cells against the direction of blood flow prior to their diapedesis is mediated by LFA-1 engaging endothelial ICAM-1 and ICAM-2 [31-33]. Further molecules such as ALCAM [34,

35], MCAM [36] and ninjuirin-1 as well as the atypical chemokine receptor 1 (ACKR1) [37] were proposed to participate in T-cell migration across the BBB during EAE and MS [38, 39] and need further exploitation.

Inhibition of immune cell trafficking across the BBB using natalizumab or sphingosine 1 phosphate receptor modulators (S1PR, currently approved: fingolimod, siponimod, ozanimod, ponesimod) has become a well-established and successful therapeutic approach for the treatment of relapsing forms of MS [40, 41]. MS patients under treatment with an anti-α4-integrin subunit mAb (natalizumab) show strongly reduced counts of adaptive immune cells, such as CD4 and CD8 T cells and B cells in their CSF [42, 43]. In addition, natalizumab reduces the number of dendritic cells in perivascular CNS spaces [44]. Taken together this supports the notion that natalizumab blocks CNS entry of both, adaptive and innate immune cell subsets, which may eventually interfere with CNS immune surveillance [45]. Although S1PR modulators limit the egress of T and B lymphocytes from the lymph nodes they reduce the numbers of T cells but not B cells in the CSF [43, 46]. This hypothesis has been supported by the observation that especially treatment with natalizumab but, to a lesser extent, also with fingolimod and dimethyl fumarate is associated with an increased risk of developing progressive multifocal leukoencephalopathy (PML), a rare but potentially fatal side effect caused by JC virus infection of glial cells [47]. As CD8 T cells are in charge of controlling CNS virus infections, development of PML is thought to be at least partly due to therapy-mediated inhibition of CNS immune surveillance by virus-specific CD8 T cells. Unfortunately, little is known about the molecular mechanisms involved in CD8 T cell trafficking across the BBB. While α4β1-integrins are essential for CD8 T cell migration into the CNS during immune surveillance and neuroinflammation similar to CD4 T cells [38], there are distinct differences in the employment of the endothelial integrin ligands from the IgCAMs between CD8 and CD4 T cells [48, 49]. Thus, exploitation of the distinct trafficking signals available at the BBB for the different immune cell subsets is a prerequisite to develop therapies that specifically block CNS entry of pathogenic T cells while leaving those required for CNS immune surveillance untouched.

Importantly, serial MRI studies have indicated that BBB dysfunction may even precede CNS immune cell infiltration and myelin damage in MS [50-52]. This is supported by our recent observation that *in vitro* BBB models derived from human induced pluripotent stem cells from MS patients show functional impairments [53]. Thus, BBB dysfunction could be the underlying cause of MS pathogenesis in some patients eventually leading to BBB breakdown later visible by MRI imaging and

ensuing neuroinflammation. In addition, continuous BBB abnormalities may also be relevant in later disease stages where histopathologically disrupted tight junctions or upregulated expression of adhesion molecules in brain microvessels were observed in inactive MS lesions as well as in normal appearing white matter of post-mortem MS brain tissue [12] [13]. Therapeutic implications are unclear since natalizumab failed to reduce disability progression using a multicomponent endpoint in secondary progressive MS, but did reduce progression in subdomains [54]. Therapeutic tightening of the BBB may provide a novel treatment option for MS that has not yet been considered. In fact, tightening of the BBB is observed as an accompanying effect under several immunomodulatory MS therapies [18], underscoring the central role of BBB dysfunction in MS pathogenesis. As experimental tightening of the BBB ameliorates clinical signs of experimental autoimmune encephalomyelitis (EAE), an animal model of MS [55], therapeutic tightening of the BBB for treating MS could be explored. This approach has been hampered until recently as studying the BBB of MS patients has been prohibited by lack of access to the tissue from patients. Recent advancements in stem cell technology have however allowed derivation of human and thus patient derived brain microvascular endothelial cells from human induced pluripotent stem cells (hiPSCs) [56-58]. hiPSC-derived *in vitro* models of the BBB have proven successful to model BBB dysfunction in inheritable neurological disorders [59-61] as well as immune cell trafficking across the BBB *in vitro* [23]. Therefore, establishing hiPSC derived models of the neurovascular unit, in which all cell types are sourced from MS patients may offer unprecedented opportunities to study somatic alterations at the level of the BBB contributing to the major hallmarks of MS pathogenesis, namely BBB dysfunction and increase immune cell entry into the CNS. In addition, these MS patient-derived *in vitro* BBB models could serve as tools for developing therapeutic strategies for BBB stabilization and for testing adverse effects of disease modifying drugs in a personalized fashion.

Contribution of the dysfunction of other outer brain barriers, e.g. the epithelial blood-cerebrospinal fluid barrier (BCSFB) surrounding the choroid plexuses or the arachnoid barrier establishing a BCSFB between the dura mater and the subarachnoid space [24] to MS pathogenesis has been largely ignored. More recent evidence for disturbance of the choroid plexus BCSFB in postmortem MS tissue [62] encourages development of MRI imaging sequences analyzing BCSFB function in MS patients. Similarly, dysfunction of the arachnoid barrier may contribute to establishment of meningeal infiltrates mainly comprised

of B cells possibly directly from the dura mater which has been identified as unique B cell niche [63, 64]; hypothetically this may allow to identify patients that respond particularly well to therapeutic targeting of B cells.

T cells

MS is considered a prototypic T cell mediated autoimmune disease based on MS associated genetic variants (single nucleotide polymorphisms, SNPs) that point to genes coding for molecules related to the activation/proliferation of T and other immune cells and due to observations made in the animal model experimental autoimmune encephalomyelitis (EAE) [3]. The classical EAE models rely on immunization with CNS-derived autoantigens emulsified in potent immune adjuvants, thereby favoring the activation of CD4 T cells and the induction of autoantibodies. Both, γ-IFN secreting Th1 and IL-17 secreting Th17 cells (summarized in [65]) and also GM-CSF producing CD4 T-cell subsets [66] have been shown to play key pathogenic roles in triggering EAE.

Different CD4 T cell subsets differ in their effector functions and trafficking profiles. In contrast to Th1 cells, Th17 cells are a heterogenous group of T cells including a subgroup of Th1-like Th17 cells expressing both, Th1 and Th17 signature cytokines and chemokine receptors. It is these Th1-like Th17 cells referred to as Th1* [67] or Th17.1 [68] that have recently been suggested to play a dominant role in MS pathogenesis. Therefore, studying the T cell profile of MS patients early in the disease may provide additional insight into potentially diverse disease triggers based on the observed alteration of distinct T cell subsets and provides a strong rationale to further explore more specific T-cell targeted therapies in early MS. As additional source of heterogeneity, studies in EAE have shown that Th17 and Th1 cells use different anatomical entry sites and distinct molecular mechanisms to reach the CNS. While Th17 cells preferentially enter the brain via the choroid plexus using the chemokine receptor CCR6 [69] and LFA-1 [70], Th1 cells preferentially infiltrate the spinal cord and require $\alpha4\beta1$-integrins for CNS entry [65, 71]. In addition a recent study has shown that the site of priming of autoaggressive T cells affects their ability to infiltrate CNS white and grey matter [72]. Exploring potential underlying T-cell subset mediated pathomechanisms in individual MS patients may thus have implications for the likelihood of response to agents like natalizumab.

In addition to CD4 T cells, there is accumulating evidence for a role of CD8 T cells in the autoimmune CNS attack in MS [73]. Indeed, CD8 T cells accumulate within white matter lesions, where they often outnumber CD4 T cells and are found in close association with oligodendrocytes and demyelinated axons [6, 74, 75]. Furthermore, CD8 T cells harboring an effector memory phenotype were shown to accumulate in the inflamed CNS of MS patients, and these cells exhibit cytotoxic and inflammatory potential as they express granzyme B and IFNγ at high frequency [38, 76]. CD8 T cells were even detected in immune cell infiltrates in cortical demyelinating lesions, which are prevalent at early stages of MS [77], underscoring an involvement of CD8 T cells at early time points of MS pathogenesis. Analyses of TCR usage by CNS infiltrating T cells provided evidence for oligoclonal expansion of CD8 but not CD4 T cells within MS lesions [78, 79] and the CSF [80], which is a likely consequence of their local MHC class-I dependent antigen-driven activation. Furthermore, recent genetic studies showed a clear association between certain MHC class I alleles and MS susceptibility [81]. Finally, results from clinical trials further underscore the contribution of lymphocyte subsets other than CD4 T cells to MS pathogenesis [82, 83]. While anti-inflammatory therapies globally targeting lymphocytes showed profound effects [84, 85], therapies specifically targeting CD4 T cells did not lead to clinical benefits in MS patients [86].

B cells

Although MS was traditionally considered as a predominantly T cell-mediated autoimmune disease, B cells are now known to be key contributors to the pathogenesis of MS. B cells, plasmablasts and plasma cells can be found in three different subcompartments of the CNS in patients with MS: the CSF, the parenchyma, and the meninges. The CSF of MS patients is characterized by the presence of oligoclonal bands in more than 90% of patients [87], and they also constitute a valuable biomarker in the diagnosis of MS [2]. The strong overlap observed between the immunoglobulin transcriptome of B cells and the corresponding immunoglobulin proteome in the CSF of MS patients demonstrated that the source of the antibodies giving rise to oligoclonal bands were clonally expanded intrathecal B cells [88]. The antigenic targets of B cells in MS patients remain unknown. Oligoclonal bands antibodies do not seem to be specific for CNS proteins but instead B cell responses are heterogeneous and include reactivity against ubiquitous self-antigens released during tissue destruction [89]. The number of B cells is usually increased in the CSF of patients with MS and the B cell to other CSF immune cells ratio was found to influence MS disease severity and progression. In this context, a high B cell to monocyte ratio in the CSF was associated

with more rapid MS progression, whereas a low ratio with predominance of monocytes was observed in patients having slower progression [90]. B cells in the CSF of MS patients are largely class-switched memory B cells and short-lived plasmablasts [91, 92].

B cells can also be found in the brain parenchyma of MS patients. Autopsy tissue studies late in the disease course indicated that the classical deep white matter perivascular demyelinating lesions of MS patients typically exhibited few B cells and plasma cells compared to the greater abundance of myeloid cells and T cells [93]. However, studies conducted in MS patients early in the disease course revealed that the CNS lesions exhibited considerable numbers of B cells as well as plasma cells, in addition to T cells and myeloid cells [94]. Altogether, these findings raise the possibility that B cells and plasma cells may be a more common feature of early parenchymal lesions.

Ectopic follicle-like aggregates containing B cells and plasma cells are found in the leptomeninges of patients with MS. Although these lymphoid structures were first described in patients with SPMS [95], meningeal aggregates of B cells are also identified in patients with RRMS and PPMS [77, 96]. The importance of these findings stems from the potential causal relationship observed between meningeal B cell inflammation and cortical damage. In this context, patients with follicle-like structures showed more pronounced subpial demyelination, microglia activation and neurite loss [97] compared to those without follicle-like aggregates. In addition, a gradient of neuronal loss from the pia mater to the white matter was observed in grey matter of patients with follicle-like structures suggesting the presence of soluble cytotoxic factors diffusing from the meninges into the grey matter [98]. The presence of ectopic follicle-like structures has been associated with more aggressive clinical disease [97, 99]. More recently, the demonstration of a specific Th17 cell subset that fosters a meningeal microenvironment that supports B-cell tropism and development highlights complex interactions of the local immune network [100]. The remarkable clinical success of B cell-depleting therapies has further underscored the importance of B cells in the pathogenesis of MS. Treatment of MS patients with rituximab and ocrelizumab results in near-complete depletion of B cells in different blood and CNS compartments [101, 102] and is associated with significant reductions in inflammatory brain lesions and clinical relapses [103]. While both memory and naïve B cells are efficiently depleted by these therapies, plasmablasts and plasma cells, which do not express CD20, are not targeted by anti-CD20 monoclonal antibodies, and hence the clinical benefit of these therapies is not related with reductions in IgM and IgG antibody levels [103, 104]. However, the effect of B cell-depleting therapies is modest in patients with progressive MS [105, 106], most likely reflecting the limited penetration of these drugs through the BBB and their difficult access to pathogenic

meningeal B-cell aggregates. This is highlighted by more recent data indicating a CNS-specific, "private" source of meningeal B-cells located in the dura mater that may or may not be potentially sourced from the nearby bone-marrow [63, 107].

Myeloid cells

The reactivation of infiltrating encephalitogenic T cells by self-cognate antigens presented by innate immune cells at CNS barriers is considered to represent a crucial step in the pathogenesis of MS. Relevant cellular elements comprise the CNS resident microglia localized in the CNS parenchyma, CNS-border associated macrophages as well as circulating dendritic cells and monocytes. Using an unbiased transcriptome approach of regionally distinct CNS-resident and circulating myeloid cells, highly dynamic and complex changes of resident and recruited circulating myeloid cells during experimental disease can be demonstrated [108]. Thus, within the innate immune cell compartment CNS-resident macrophages undergo clonal expansion but are functionally dispensable for antigen presentation. In contrast, dendritic cells and monocyte-derived cells appear to be crucial for antigen presentation. Therefore, even among myeloid cells there is a high degree of dynamic subtype specification dependent on neuroinflammatory disease stage and anatomical compartment. A high degree of plasticity is also observed when focusing solely on CNS resident microglia. Several (conditional) transgenic mouse models indicate disease phase specific roles of microglia with crucial disease promoting effector functions but also phagocytic clearance of tissue debris relevant in the recovery phase [109-111]. Necroptosis of proinflammatory microglia and repopulation to a regenerative state was demonstrated to drive remyelination in toxic and inflammatory CNS demyelination [112]. Therefore, targeting proinflammatory microglia may appear attractive both to modulate white matter inflammation and support remyelination. However, although considerable progress in the understanding of myeloid cell plasticity has been achieved, we are still not capable of exploiting functional and anatomical heterogeneity therapeutically [113, 114]. More recently, inhibitors of Bruton's tyrosine kinase (BTK), a key signaling element for both B cells and myeloid cells demonstrated efficacy especially on MRI parameters of focal inflammation in phase II clinical trials [115, 116]. The hypothesis, that brain penetrant BTK-inhibitors may also target chronic, microglia-associated inflammation in chronic progressive MS is currently being explored in clinical phase III trials.

Biomarkers of disease heterogeneity and individualized treatment responses

Biomarkers are particularly relevant in disorders characterized by a high degree of heterogeneity, like MS, for which it is expected that biomarkers will fully capture the different aspects of disease heterogeneity and help in better disease diagnosis, stratification and monitoring [117]. Among the different categories of biomarkers that have been described in MS, treatment response biomarkers constitute the cornerstone for the development of a personalized therapy in the disease. While treatment response biomarkers in the strict sense refer to those associated with the clinical response to therapies, the vast armamentarium of therapeutic options existing nowadays for MS patients certainly opens the potential benefits of this type of biomarkers to include guidance on dose adjustment, switch and escalation therapies, remission induction, safety monitoring and risk stratification. The ultimate goal of personalized therapy is to administer treatment to the patient who is going to respond to it and will not develop adverse drug effects. The clinical use of treatment response biomarkers may differ depending on the nature of biomarkers. Treatment response biomarkers of DNA or RNA nature can be identified by the use of *omics* technologies (this is the field of "pharmacogenomics") such as genomics and transcriptomics. Ideally these biomarkers can be determined before receiving treatment or during the first months of treatment, to make predictions of the response of each particular patient to the drug. After a follow-up, patients will be classified according to clinical and / or radiological criteria allowing to calculate the predictive accuracies of treatment response biomarkers by comparing the initial predictions made before treatment or in the first months of treatment with the real response phenotypes observed during follow-up [118]. Unfortunately, there are no examples of treatment response biomarkers that are used in routine MS clinical practice.

A different scenario is observed for biomarkers of protein nature, which are more suitable for therapy monitoring, pharmacodynamics, and as surrogate endpoints [117]. A promising biomarker that is gradually being implemented into MS clinical practice for therapy monitoring is the blood neurofilament light subunit [119]. Blood neurofilament light levels are modified by the effect of MS therapies and are associated with treatment response, and have the potential to be used as informative endpoint in phase II clinical trials [120]. Interestingly, serum neurofilament levels have also been proposed as a biomarker for early identification of PML in MS patients receiving treatment with natalizumab [121, 122].

Treatment of acute MS relapses: a paradigm for individualized therapy?

MS relapses lead to sustained residual disability in more than 40% of the patients [123]. Acute therapy follows a stereotypic approach with administration of intravenous, high dose intravenous glucocorticosteroids (GC, methylprednisolone); relapses not responsive to GC can subsequently be treated using apheresis techniques (plasma exchange, immunoadsorption) [124]. Therapy in this situation is far from "personalized" with few, mainly clinical predictors of therapy response to GC or apheresis (Figure 1). GC exert pleiotropic anti-inflammatory effects, e.g. by inhibiting secretion of pro-inflammatory mediators (Th1 associated cytokines, chemokines, antibodies, nitric oxide), effects on activation and migration of different immune cells (T cells, macrophages, microglia), increase of CD25+, FoxP3+Treg cells, and effects on adhesion molecules and tight-junction proteins in endothelial cells [125, 126]. Lack of response in a proportion of patients [127] may point to distinct pathomechanisms incompletely targeted by repeated and even dose escalated GC. Thus, in a cohort study of MS patients that had undergone brain biopsy, treatment response to apheresis in the context of steroid-resistant relapse was associated with distinct histopathological characteristics [128]. Those patients where early demyelinating CNS-lesions were characterized by deposition of immunoglobulin and complement responded relatively well to apheresis therapy. This was in contrast to patients with lesions characterized by changes reminiscent of primary oligodendrocyte damage [129]. Together with the concept of interindividual histopathological heterogeneity with intraindividually stable phenotypes this potential interference of apheresis therapy with antibody- and complement-mediated demyelination could hypothetically be an element of individualizing treatment algorithms [130, 131]. However, this concept is controversially discussed, and other confounding factors may be relevant in this highly selected cohort of patients that underwent brain biopsy, a procedure which is very rarely performed in the differential diagnosis of MS.

GC resistance may involve additional or alternative dynamic mechanisms, e.g. alterations of the glucocorticosteroid receptor (GR) complex [132]. In MS, a different intracellular distribution of the GR and its molecular chaperone, heat shock protein 90 (hsp90) was demonstrated in GC-resistant patients [133]. Alterations of GR isoform expression and DNA-binding was reported in experimental MS models and in other non-CNS disease models [134, 135]. Regulation by specific microRNAs, proteasomal degradation as well as specific interleukins, which regulate transcription factors interfering with GR signaling may also play a role [136]. In an attempt to increase glucocorticosteroid effects, synergistic effects of vitamin D and other mTOR inhibitors with glucocorticosteroids were demonstrated in experimental models of multiple sclerosis [137]. Signaling via mTOR (mammalian/mechanistic target of rapamycin), an evolutionary highly conserved

serine/threonine kinase crucial also during autoimmune neuroinflammation appears to be an important modulator of GC response [138, 139], and mTOR inhibition via vitamin D and also other specific mTOR inhibitors already in clinical use (e.g. everolimus) increased GR expression/GC-mediated anti-inflammatory effects [137]. Thus, if clinically validated, approaches to increase GC efficacy via enhancement of GR-signaling may reduce the need for costly and risk-prone apheresis procedures. As high dose GC therapy is also first line treatment in a variety of other neuroinflammatory diseases of different etiology (e.g. CNS vasculitis, antibody mediated encephalitis), these approaches may also have implications beyond acute MS relapses [137]. Severe deficiency of serum vitamin D was associated with a higher likelihood of a GC-resistant MS relapse. However, currently there are no marker profiles that could guide "precise" choice of treatment in this context (Figure 1) [140].

Conclusions and future perspectives

Despite major progress in the treatment of MS, there is an unmet need to tailor therapeutic approaches, which currently mainly rely on clinical, partly subjective parameters. Heterogeneity in pathomechanisms, changes over time also against the backdrop of aging not well appreciated by static scientific approaches and interindividual differences that govern pharmacodynamic and pharmacokinetic characteristics of pleiotropic substances are only some aspects that highlight the opportunities but also complexity of "individualized" therapeutic approaches (Figure 2). More recent experimental findings underscoring that the site of T-cell priming affects T cell trafficking and effector properties and thus immunopathology in the CNS open up the possibility of peripheral site directed treatment approaches (e.g. skin or gut) to modulate T-cell pathophysiology at remote sites [72]. The challenges to further unravel pathophysiological heterogeneity and subsequent personalized treatment strategies in MS are high. Nevertheless, experience with related disorders such as neuromyelitis spectrum disorders (NMOSD) and more recently also with myelin-oligodendrocyte antibody disease (MOGAD) has paved the way for further progress. Here, translational research into syndromes that clinically partially overlap with MS has elucidated distinct pathophysiological mechanisms which has led to rapid advancement of specific therapeutic strategies [141].

However, despite this progress in many fields we must not forget that for MS societal and political factors govern access to medication, and unforeseen developments such as the global Covid 19 pandemia may at least temporally outweigh attempts to tailor therapy on an individual level.

Conflict of interest

Author contributions

All authors were involved in conceptualization, writing, and revising the context.

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Conflicts of interest

Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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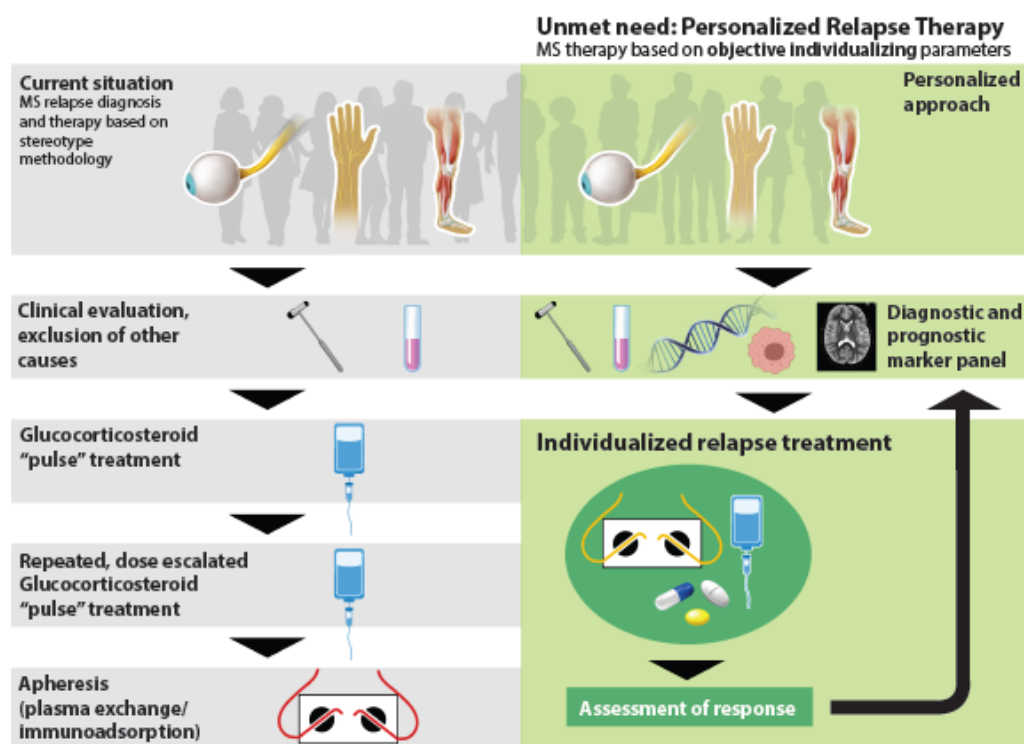
Legends to figures

Figure 1: Unmet needs of personalized treatment approaches: MS relapse

(Left) MS standard treatment. With the occurrence of new or worsening symptoms of MS (e.g. visual, sensory, motor), further workup and therapy currently follow stereotypic flows and guidelines. Following neurological examination and exclusion of confounding factors (e.g. infections), high dose glucocorticosteroid "pulse therapy" is the current standard of care. If symptoms do not remit, a second, potentially dose-escalated glucocorticosteroid pulse is administered. Apheresis techniques (plasma exchange/immunoadsorption) are employed in glucocorticosteroid-resistant cases, not taking into account different sources of heterogeneity that lead to treatment non-response ("try and error"). (Right) MS personalized treatment. In a personalized approach, objective and prognostic markers on an individual level would be taken into account for a treatment decision. This marker profile would be informed by the mechanisms associated with heterogeneity in response to GC therapy as discussed in the text. Treatment could consist of different approaches (e.g. glucocorticosteroids, apheresis, new substances) also in combination. It is unclear, if treatment response in acute relapses varies over time, so marker profiles would potentially have to be individually updated to ensure response state during future relapses. Although some current findings will inform further research, at present no such marker profile exists.
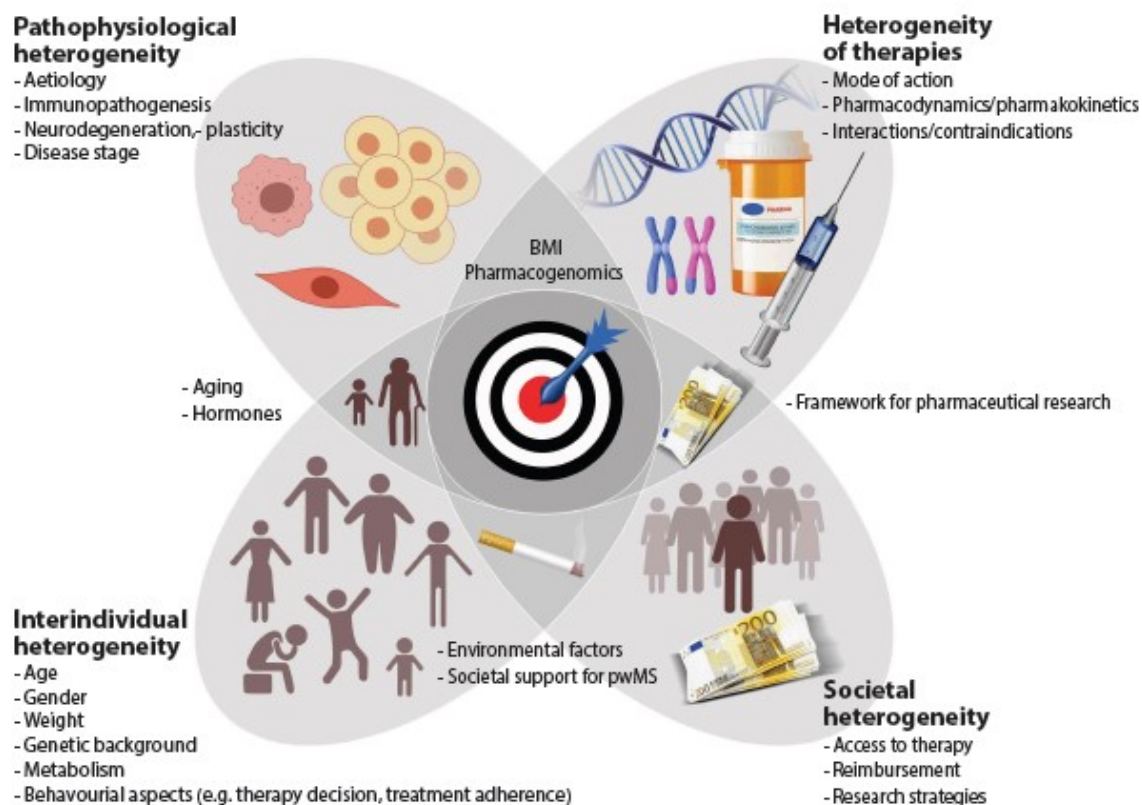
Figure 2: Factors that determine heterogeneity of treatment response in MS Individual factors (e.g. genetic background) combined with inter-individual heterogeneity of immunopathophysiological changes as detailed in the text. Together they determine response to immunotherapy, which in itself harbours multiple sources of heterogeneity. Finally, societal aspects are relevant both in terms of MS pathophysiology (e.g. environmental risk factors), development of and access to treatment as well as support to cope with the disease. BMI: body mass index.

Graphical abstract text

Currently multiple sclerosis diagnosis and therapy follows stereotype guidelines, where in a "try and error" approach patients are divided into treatment responders or non-responders. In this review, we discuss different immunopathophysiological sources of heterogeneity; still an unmet medical need, ideally these sources of heterogeneity would be the basis for individualized disease management decisions.