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Advancing Trial Design in Progressive Multiple Sclerosis

Targets of therapy in progressive MS

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***Abstract*:** Highly effective anti-inflammatory therapies have so far been developed for patients with relapsing/remitting multiple sclerosis, which also show some benefits in the early progressive stage of the disease. However, treatment options for patients, who have entered the progressive phase, are still limited. Disease starts as an inflammatory process, which induces focal demyelinating lesions in the gray and white matter. This stage of the disease dominates in the relapsing phase, extends into the early stages of progressive disease, and can be targeted by current anti-inflammatory treatments. In parallel, inflammation accumulates behind a closed or repaired blood brain barrier, and this process peaks in the late relapsing and early progressive stage and then declines. Some data suggest that this process may be targeted by immune ablation and hematopoietic stem cell transplantation. In the late stage, inflammation may decline to levels seen in age-matched controls, but age and disease burden–related neurodegeneration ensues. Such neurodegeneration affects the damaged brain and spinal cord, in which functional reserve capacity is exhausted, giving rise to further disability progression. Anti-inflammatory treatments are unlikely to be beneficial in this stage of the disease, but neuroprotective and repair-inducing strategies may still be effective.

Keywords: Multiple sclerosis, inflammation, neurodegeneration, treatment

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Introduction

Anti-inflammatory and immunomodulatory therapies are highly effective in the early relapsing stage of multiple sclerosis (MS), but with few exceptions they have failed to show a beneficial effect, when patients entered the progressive stage. For this reason a widely held concept is that MS starts as an inflammatory disease, but is driven at later stages by neurodegeneration, which develops independently from inflammatory mechanisms. This view in part contradicts neuropathological experience, which shows that inflammation, defined by T- and B-cell infiltrates, is invariably associated with active demyelination and tissue injury in the progressive stage of the disease.[1] In this short review, we discuss the neuropathological differences between relapsing and progressive MS, the current knowledge of pathophysiological mechanisms driving tissue injury in progressive MS, and the implications of these findings for currently established and future treatments of patients.

Neuropathological features distinguishing relapsing from progressive MS

The neuropathological changes in the brain of patients with relapsing or progressive MS are essentially similar. Inflammation, signified by the presence of T and B lymphocytes, is present, and this is associated with the formation and/or expansion of focal lesions of primary demyelination in the white and gray matter and with neurodegeneration in the plaques and in normal-appearing white and gray matter. Focal lesions are characterized by profound astrocytic scar formation and a variable extent of axonal loss and remyelination. However, the relative incidence of different lesion features changes with time of disease evolution.[2] New focal white matter lesions dominate the pathological picture of early MS, and many of the plaques are in the active stage of demyelination. In contrast, in the progressive stage, new active lesions become rare, but many of the focal lesions display a rim of activated microglia at the border and some macrophages with recent myelin degradation products. This suggests

slow expansion of pre-existing lesions.[3] In addition, cerebral and cerebellar cortical demyelination, which is present but sparse in the early stage of MS, becomes very prominent in the progressive stage, reaching in some extreme examples an extent of up to 90%.[4–6] In addition, profound diffuse pathology is present in the normal-appearing white and gray mater, which consists of small perivenous inflammatory infiltrates, surrounded by small rims of demyelination, diffuse astrocytic gliosis as well as diffuse microglia activation and axonal degeneration.[4,7] Neuronal loss is pronounced in cortical lesions,[8] and the extent of diffuse pathology in the normal-appearing white matter correlates better with the extent of cortical than white matter demyelination.[4] Diffuse neurodegeneration in the gray as well as the white matter seems to be driven in part by the inflammatory process in the leptomeninges as well as by anterograde and retrograde degeneration resulting from axonal loss in focal lesions.[9,10]

Inflammatory infiltrates in MS are dominated by $CD8^+$ T lymphocytes, CD20 positive B cells, and immunoglobulin-producing plasma cells.[1,11,12] $CD8^+$ T cells, B cells, and plasma cells show clonal expansion, which indicates their activation by specific cognate antigen(s) within the central nervous system (CNS).[13,14] $CD8^+$ T cells dominate the inflammatory reaction not only in MS but also in most other inflammatory diseases in the human CNS, in particular in virus-induced encephalitides. In contrast, B cells are enriched within MS lesions and the B cell/monocyte ratio in the cerebrospinal fluid (CSF) correlated with the severity of disease progression.[15] The patterns of inflammation are similar between relapsing and progressive MS, although the global extent of lymphocytic inflammation is higher in acute or relapsing MS in comparison to progressive MS.[1] Phenotypic characterization of B cells in MS lesions has so far not been performed but in the CSF short-lived plasmablasts dominate.[16] $CD8^+$ T cells express markers of either activated cytotoxic T cells (granzyme B expression[17]) or of tissue-resident effector memory T cells. A major difference between acute/relapsing MS and progressive MS is that in the former, the lymphocytic infiltration is associated with profound blood–brain barrier damage, while in the progressive stage, inflammation is at least partly compartmentalized in the brain behind an intact (possibly repaired) blood–brain barrier.[18]

Active tissue injury, consistent of demyelination, axonal transsection, and neuronal degeneration, is associated with profound microglia activation.[19,20] In addition, however, microglia is already partly activated toward a pro-inflammatory phenotype in the normal white matter of controls, and this is even more the case in the normal-appearing white matter of MS patients.[21] Global pro-inflammatory microglia activation increases with age of controls and with age and disease duration in MS patients. In areas of active tissue injury, microglia are dominantly activated into a pro-inflammatory phenotype, expressing functional markers for oxidative activation, phagocytosis, and antigen presentation. In active lesions of acute and relapsing MS, the lesions are additionally infiltrated by recruited macrophages, which contribute to about 60% of the global macrophage population in the lesions.[21] In response to myelin phagocytosis, these macrophages convert to an intermediate phenotype, co-expressing pro- and anti-inflammatory markers.[22] This coincides with the induction of remyelination in the lesions. This is different in slowly expanding lesions of progressive MS, where active tissue injury is mainly associated with pro-inflammatory microglia activation, macrophage recruitment is sparse, and expression of anti-inflammatory markers is minimal to absent.[21]

Perivascular and meningeal inflammation may occur as lymphocytic aggregates, which may show features of tertiary lymph follicles.[23] One of their prominent features is the high content of B lymphocytes. Although they are already present in the earliest stages of MS,[24] their number and incidence increase with disease duration, reaching highest levels at the early phase of progressive disease.[4–6] Patients who have such follicle-like inflammatory aggregates in the meninges have a more aggressive progressive disease, reduced life expectancy, and in pathology more profound cortical (subpial) demyelination and diffuse brain injury in the normal-appearing white and gray matter.[25]

The inflammatory process appears to die out in late stages of progressive MS. In such patients, lymphocytic infiltrates are reduced to very low levels, similar to those seen also in age-matched controls. Active demyelination is absent in the brain of these patients, but there is a low level of ongoing (axonal) neurodegeneration, which too is similar to that present in age-matched controls.[1]

Mechanisms of demyelination and tissue injury

A broad spectrum of different mechanisms of immune-mediated tissue injury has been identified in experimental models, which were suggested to be relevant for MS pathogenesis.[26] Many of these mechanisms are however shared between different inflammatory brain diseases, which do not show the MS typical features of inflammatory primary demyelination with relative axonal sparing. Comparing active MS lesions at different stages of their evolution and with other inflammatory or

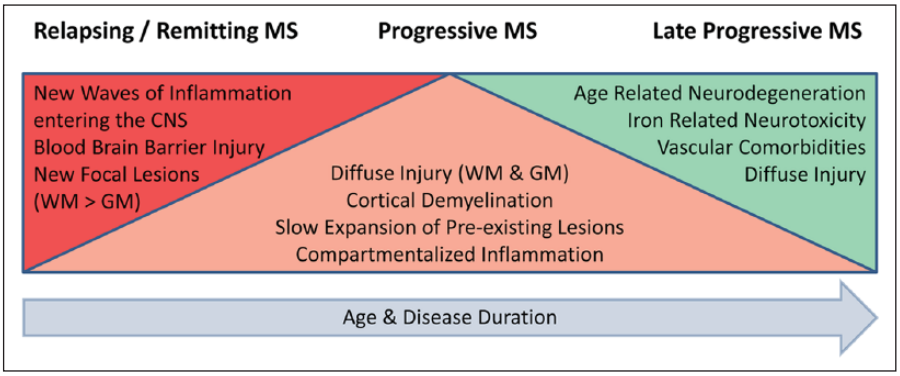

Figure 1. Pathological substrates of MS in different disease stages.
WM: white matter, GM: gray matter.

non-inflammatory diseases, a dominant pathway of tissue injury appeared, which involves microglia activation, their production of reactive oxygen and nitric oxide species, and profound oxidative injury of oligodendrocytes, axons, and neurons, in particular when they show changes of initial damage or cell death.[27,28] Mitochondria are particularly vulnerable in conditions of oxidative injury and it is, thus, not surprising to see extensive mitochondrial damage in active stages of demyelination and neurodegeneration[29,30] and a chronic mitochondrial dysfunction due to mutations and deletions of mitochondrial DNA in progressive MS.[31] The consequence of mitochondrial injury is energy deficiency, best described by the terms histotoxic or virtual hypoxia.[32] Downstream consequences of oxidative injury, mitochondrial damage, and energy deficiency are endoplasmic reticulum stress and neurodegeneration due to ionic imbalance, excitotoxicity, and intracellular calcium accumulation.[33]

These mechanisms are very prominent in the MS brain and apparently initiated by the chronic inflammatory process. However, quite similar mechanisms also play a role in brain aging, age-related neurodegenerative diseases, and vascular diseases. The latter is particularly important, since recent data from pathology and imaging indicate that MS lesions may arise at any sites of the brain, but persistent lesions with extensive axonal loss and lack of repair mainly accumulate in brain areas with low vascular perfusion and oxygen tension.[10,34] Thus, age-related neurodegeneration, low vascular perfusion in the normal brain, and vascular co-morbidities in aging patients amplify tissue damage and neurodegeneration in MS.

Finally, the normal human brain progressively accumulates iron with aging, and this global iron accumulation appears to be amplified in MS patients.[35] Iron mainly accumulates in myelin and oligodendrocytes, and oligodendrocyte death in MS lesions liberates iron from the intracellular stores. Free divalent iron potentiates oxidative injury through the formation of highly reactive hydroxyl radicals. Thus, iron-related neurodegeneration is an additional factor, which amplifies tissue injury and neurodegeneration in the progressive stage of MS.

The evolution of brain damage in MS may require stage-dependent therapeutic strategies

Overall, on the basis of pathology, MS can be roughly categorized into three different disease stages (Figure 1): an initial (early stage) of brain injury driven by systemic inflammation, a second stage of compartmentalized inflammation in the brain and spinal cord, and a last phase of inflammation independent, but age and disease burden–related neurodegeneration. It is likely, but not yet formally, proven that all stages of the disease are triggered by the initial inflammatory response and, thus, effective anti-inflammatory treatment in early MS should reduce or even abrogate progression in the subsequent disease stages. In addition, it has to be acknowledged that there is no strict separation of these disease stages but that the respective pathogenetic mechanisms in part act in parallel (Figure 1).

Early inflammatory stage

The first stage of inflammation driven by the systemic immune reaction gives rise to new focal lesions dominantly located in the white matter. This dominates in patients in the early relapsing/remitting stage of the disease but extends into the early stages of (primary and secondary) progressive MS. In the latter patients, disease progression may still be associated with some clinical disease activity and/or the appearance of some contrast-enhancing lesions in magnetic resonance imaging (MRI). Anti-inflammatory or immunomodulatory treatment is

effective in such patients with progressive disease, and recent trials show that this is also associated with a moderate reduction of disability progression (ocrelizumab;[36] siponimod: Novartis release on BAF312).

Compartmentalized inflammation in progressive MS

A dominant feature of the pathology of progressive MS is the presence of a compartmentalized inflammatory response, where T cells, B cells, and plasma cells are trapped within the brain and spinal cord behind a closed or repaired blood–brain barrier. This gives rise to large inflammatory (follicle-like) aggregates in the meninges and large perivascular spaces, associated with active cortical demyelination, slow expansion of pre-existing white matter lesions, and diffuse injury of the normal-appearing white and gray matter. Although trapped inflammation builds up already in the early stage of the disease, it reaches its peak in the late relapsing and early progressive phase. The therapeutic strategy in this stage of the disease should be the blockade of the inflammatory response within the CNS. To be effective, respective anti-inflammatory drugs have to reach the inflammatory response behind a closed blood–brain barrier. Thus, most of the current biological drugs (such as antibodies) will not reach their specific target in the brain in sufficient concentrations. Furthermore, blockade of leukocyte recruitment from lymphatic tissue or their migration through the blood brain barrier, which can be achieved by sphingosine phosphate receptor or α4 integrin blockade,[37,38] is unlikely to be effective, when the immune cells are already within the CNS compartment. Since T cells in the lesions in progressive MS show only a low degree of activation and a very low rate of proliferation, classical immunosuppressive treatments are not a prime therapeutic option, even when they can get access to the brain. Furthermore, as discussed above, a major population of $CD8^+$ T cells within the lesions of progressive MS displays a phenotype of tissue-resident effector memory cells. To become tissue-resident cells such T lymphocytes downregulate their expression of sphingosine phosphate receptors[39] and thus, they can also no longer be targeted by drugs like fingolimod or siponimod. Unfortunately, so far, very little is known about strategies to therapeutically target tissue-resident T or B lymphocytes. Whether intrathecal elimination of T and/or B cells slows disease progression is currently unclear.

However, immune ablation with subsequent bone marrow (stem cell) transplantation may have an effect on compartmentalized inflammation in the brain. It has been applied in patients with severe progressive disease, and the therapeutic effects seem to be more pronounced compared to conventional immunosuppressive or immunomodulatory treatments.[40,41] Furthermore, aggressive immune ablation uses a combination of drugs, which have the potential to get access to the CNS through the blood brain barrier, shown by a short-term increase in brain atrophy, possibly due to direct cytotoxic actions.[42] Neuropathological studies on a very small number of patients showed a profound reduction of the inflammatory response, but there was some residual inflammation and microglia activation associated with persistent demyelination or neurodegeneration.[43] However, these neuropathological data mainly came from patients, who died early after immune ablation and bone marrow transplantation, and data on the long-term effects of this treatment on inflammation in the brain and spinal cord are sparse. Despite these caveats, new MRI data indicate that after the acute phase following immune ablation and bone marrow transplantation, the rate of brain atrophy declines to levels seen in age-matched controls.[40]

Late stage of progressive MS

In the last stage of MS, progressive neurodegeneration occurs even in the absence of an overt inflammatory response.[1] The disease mechanisms in this stage of the disease seem to be similar to those in brain aging, but they occur in a brain and spinal cord, which is already damaged beyond the stage of functional compensation.[10,33] Since microglia activation, oxidative injury, mitochondrial damage, and subsequent "virtual hypoxia" are important amplification factors of neurodegeneration in chronic inflammation as well as aging, these neurodegenerative mechanisms are important drivers of disease in all stages of MS. Therapeutic goal for this stage of the disease (or this type of injury) should be both the induction of functional improvement and the reduction of the speed of neurodegeneration. Thus, a treatment trial may have highly ambitious goals, such as the rate of patients with short-term clinical improvement and long-term halt of disease progression. This has recently been shown in a small controlled trial in patients treated with high-dose biotin,[44] which seems to counteract the state of energy deficiency in "virtual hypoxia." In addition, some data suggest that the progression of neurodegeneration may be ameliorated by simvastation[45] and siponimod (Novartis press release on BAF312). The mechanisms behind the effects of the latter drugs in MS are not fully understood at present.

An alternative strategy is the stimulation of remyelination and repair either through pharmacological

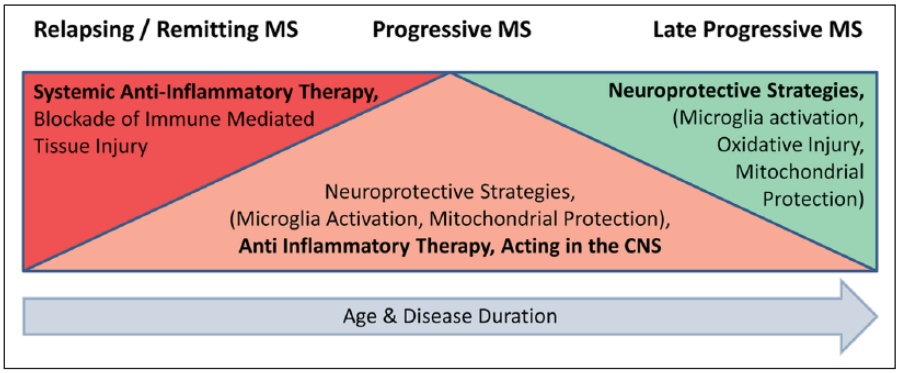

Figure 2. Therapeutic strategies in different stages of MS evolution.

approaches or through cell transplantation. Remyelination in demyelinated lesions may result in functional improvement and in neuroprotection, as shown in experimental animals.[46] However, the reasons for remyelination failure in MS lesions are highly complex and not only involve the loss of oligodendrocyte progenitor cells or their blockade of differentiation into myelinating cells, which are mechanisms that can be targeted pharmacologically or by cell transplantation. Important additional factors are recurrent inflammatory demyelination in remyelinated areas,[47,48] extensive loss and functional impairment of axons in chronic demyelinated lesions,[49] and impairment of the regenerative capacity due to age-related factors and vascular comorbidities.[10] Furthermore, spontaneous remyelination occurs in MS patients and lesions, its extent being variable in different patients and dependent upon lesion location.[50,51] It is thus expected that therapies stimulating remyelination will only be effective in combination with anti-inflammatory treatments and in lesions, which still contain sufficient axons to be remyelinated. Furthermore, paraclinical markers, which determine the extent of spontaneous and treatment-induced remyelination and which define the reasons for remyelination failure, are urgently required for the design of respective clinical trials.

Conclusion

In this review, it is discussed that treatment targets are different in different stages of disease evolution in MS patients (Figure 2). However, it is important to consider that the mechanisms, which dominate in a given disease stage are also involved in the other stages. For pragmatic reasons, it may be useful in clinical trials to define the effect of anti-inflammatory versus neuroprotective treatments in those disease stages, where the respective mechanisms dominate, but when a positive treatment effect is proven, it is likely that they are also in part effective during other disease stages.

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