Immunopathological Factors Associated with Disability in Multiple Sclerosis

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ABSTRACT

In multiple sclerosis (MS), disability occurs as a result of complex interactions between glial cells and diverse components of acquired immunity. Microglial cells significantly contribute to disability progression by re-activating infiltrating lymphocytes, releasing neuro- and oligodendrocytotoxic mediators, inducing oxidative stress, and interfering with neuroplasticity. Recent research has emphasized the significance of meningeal B cell infiltrates in development of cognitive decline and disability. B cells are also involved in disability progression by way of production of neurotoxic antibodies directed against axoglial

antigens. Among several immunological factors, neurofilament light chain antibodies, IgM-type oligoclonal bands, complement factor C3, and microglia-derived mediators stick out as potential reliable predictors of disability progression in MS patients. Better understanding of the interactions between innate immunity and neuroaxonal degeneration may result in development of novel and effective therapeutics for progressive types of MS.

Keywords: Multiple sclerosis, antibody, microglia, cytokine, disability

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disorder of the central nervous system (CNS), characterized with immune infiltration, demyelination and axonal loss (1). Most MS patients present with relapsing-remitting MS (RRMS), which in due course transforms into secondary progressive MS (SPMS), characterized with irreversible axonal loss, neurodegeneration and permanent disability (2). Transition to the progressive stage is typified with suppression of new inflammatory activity, reduction of T cell density and enhancement of widespread gliosis in the CNS (3, 4). Although oxidative stress, mitochondrial dysfunction and disturbed remyelination are major hallmarks of progressive MS and inflammatory activity may not be evident with common imaging methods, inflammation never completely ceases to exist in the CNS (3–6). There is evidence suggesting that cognitive deterioration in MS is driven at least partially by meningeal infiltrates (7). Moreover, under pathological conditions, the primary innate immunity cells of the CNS, microglia gain a pro-inflammatory phenotype (M1) and disturb neurons, oligodendrocytes and the blood-brain barrier (BBB) (8). Natural killer (NK) cells, NK-T cells, dendritic cells, gd-T cells and mast cells are additional innate immunity components that are involved in progression of MS symptoms (9). Several humoral factors including antibodies, complement factors, cytokines and chemokines significantly contribute to neuroaxonal damage and subsequent disability. It is well-

known that proinflammatory cytokines and mediators released by microglial cells activate lymphocytes and macrophages and in turn these immune cells release humoral mediators that enhance microglial activity (8–10). Thus, in advanced stages of MS, a pro-inflammatory feedback loop is presumably established among M1 microglia, T helper (Th) 1 cells, Th17 cells, macrophages and other innate immunity cells ultimately culminating in permanent disability.

Glial Activity and Disability

The CNS phagocytizing residents, microglia, can exert toxicity against neurons and oligodendrocyte precursor cells and reactivate the CNS-infiltrating T cells by releasing matrix metalloproteinases, inflammatory cytokines (e.g., IL-6, IL-1b, TNF-a), glutamate, nitric oxide synthase and free radicals (reactive oxygen and nitrogen species) especially when they convert to the pro-inflammatory M1 phenotype (8, 11). Microglial cells are also capable of presenting CNS antigens to lymphocytes (12). By contrast, the anti-inflammatory M2 microglia promote axonal regeneration and remyelination by releasing immunosuppressive (e.g., IL-10) and neurotrophic factors (e.g. insulin-like growth factor-1, brain-derived neurotrophic factor, ferritin) (8, 13). They also phagocytize debris and remove inhibitory extracellular molecules thereby enabling remyelination (14). Notably, adoptive transfer of M2-polarized cells attenuates the clinical severity in the animal model of MS, experimental

autoimmune encephalomyelitis (EAE) (15). Thus activation of M1 microglia is an important step forward in disability progression. Enhanced pro-inflammatory microglial activity has been shown to cause astrocyte dysfunction, disrupt the BBB, increase lymphocyte/macrophage recruitment to the CNS, reduce neuroplasticity, interfere with remyelination and enhance oxidative stress and mitochondrial dysfunction (8, 10). Therefore, unsurprisingly, perivascular microglia clusters in the cortical gray matter have been associated with disability progression in EAE studies (16).

Enhancement of microglial activity in MS is primarily achieved through activation of pathogen recognition receptors such as toll-like receptors (TLR) and nod-like receptors (NLR). These receptors are not only activated by pathogen-derived molecules such as lipopolysaccharides but also by ATP molecules and damage-associated molecules such as high mobility group box 1 (HMGB1), the levels of which are elevated in MS due to neuronal disturbance. Activation of these receptors trigger many intracellular inflammatory pathway molecules (e.g. NFkB, NLRP inflammasome complex) giving rise to the release of pro-inflammatory cytokines and other toxic mediators (17, 18).

Chitinase-3-like protein 1 (CHI3L1, also known as YKL-40) is a secreted glycoprotein produced by a variety of cells including microglia and astrocytes (19). Elevated cerebrospinal fluid (CSF) levels of CHI3L1 are associated with increased likelihood of conversion from clinically isolated syndrome (CIS) to RRMS. Moreover, increased CSF CHI3L1 levels in RRMS patients are associated with increased disability scores [paced auditory serial addition test (PASAT) and expanded disability status scale (EDSS)] in MS and conversion to SPMS (20), thus providing firm evidence for the involvement of glial cells in clinical MS progression.

Pro-inflammatory cytokines released by microglia interact with neuronal function and cause disability through various different pathways. Among these, an interesting mechanism of action is disturbance of neuroplasticity, which is highly required for preservation of normal CNS functions in health and disease (21). EAE studies have shown that pro-inflammatory cytokines such as IL-6 and IL-1b are capable of altering neurotransmitter release and neurotransmitter receptor expression. For instance, inhibition of cytokine production by siponimod has attenuated glutamatergic and GABAergic synaptic transmission in a mouse model of EAE (22). Moreover, studies on brain tissues of MS patients have shown IL-1b-induced glutamate-mediated excitatory postsynaptic currents potentially causing neurodegenerative damage and disease progression (23). On the other hand, cytokines IFN-g and TNF-a activate enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) and thus generate enhanced tryptophan-kynurenine pathway activity. Byproducts of this pathway, quinolinic acid and kynurenic acid, interfere with synaptic neurotransmitter receptor functions. Levels of neurotoxic kynurenine metabolites are increased in the CNS of animals with EAE and kynurenine inhibitors ameliorate clinical symptoms of EAE suggesting involvement of tryptophan-kynurenine pathway in disability progression of MS patients (24, 25). In brief, microglial activation induces increased glutamatergic toxicity through enhancement of tryptophan-kynurenine pathway activity, increased release of cytokines and reduction of astrocytic glutamate transporter expression ultimately causing neuronal and oligodendrocytic death and disability.

Another important mechanism by which microglia cause enhanced disability is increased oxidative stress. In line with this view, antioxidant reagents have been shown to improve the clinical and immunological features of EAE induced by myelin peptide immunization. 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) is a membrane permeable free radical scavenger, which preserves BBB integrity, decreases macrophage and T cell infiltration and lessens TNF-a and IFN-g production by immune cells, whereas MitoQ, is a mitochondria-targeted

antioxidant, which ameliorates neurological disability and suppresses inflammation in the CNS (6). Amelioration of EAE after treatment with both reagents is coupled with alteration in redox status and reduction in microglial activity strongly emphasizing the link between oxidative stress status and neuroinflammation.

More direct evidence about the association between microglia and disability in MS stems from treatment studies with microglia inhibitors. Treatment with masitinib, a potent and selective tyrosine kinase inhibitor, induced an improvement in the MS functional composite scores of progressive MS patients (26). Similarly, treatment with ibudilast, a phosphodiesterase inhibitor, conferred protection against neurological disability and brain atrophy in progressive MS patients (27). Finally, another microglia inhibitor minocycline has shown a preventive effect against conversion from CIS to MS (28).

Astrocytes have been shown to display immunological functions and act as an innate immunity component under inflammatory conditions (29). Microglial activation results in astrocyte dysfunction characterized with reduced extracellular glutamate buffering activity, which leads to increased neuronal excitation, increased oligodendrocyte loss, impaired remyelination and disrupted BBB integrity (30). CSF levels of astrocytic glial fibrillary acidic protein (GFAP) directly correlate with EDSS scores of RRMS patients. MS patients with higher GFAP levels gain an EDSS score of 3.0 earlier than those with lower GFAP levels (31). Astrocytes are known to produce anti-inflammatory cytokines IL-10 and IL-27, which reduce Th1- and Th17-type immune cell levels and promote remyelination. Disruption of astrocyte functions due to increased M1 microglia activity might potentially exert a negative impact on these two functions in MS (32).

Meningeal Inflammation and B Cells

Although MS lesions primarily contain autoreactive T cells and T cells are main drivers of the disease, the beneficial effects of B cell depleting therapies on reversing disability progression have implicated the significance of B cells in MS immunopathogenesis. B cells contribute to MS induction via antigen presentation, pro-inflammatory cytokine and autoantibody production (33, 34). Contribution of demyelination in cortical and deep gray matter to disability progression has become increasingly recognized. Cortical demyelination is particularly associated with cortical atrophy and cognitive impairment (35). Recently, B cell aggregates resembling tertiary lymphoid organs (TLOs) were discovered in the meninges of MS patients and these structures were found to be associated with cortical MS lesions (36).

In MS, presence of oligoclonal bands that are only found in CSF, detection of an intrathecal polyspecific antiviral immune response against-measles, rubella and varicella zoster viruses (MRZ reaction) and demonstration of B cells undergoing somatic hypermutation all suggest that differentiation of autoreactive B cells may at least partially occur inside the CNS (34). Moreover, autoreactive B cells are known to appear in the CSF of MS patients in the very early stages of the disease and conversion of CIS to MS is associated with increased memory B cell levels in the peripheral blood further emphasizing the significance of B cells in MS (37). Presence of meningeal aggregates and their association with disease severity provide further evidence for involvement of B cells in MS. Meningeal B cell infiltrates and TLOs often emerge in close proximity to cortical subpial lesions and are associated with cognitive deterioration, brain atrophy, neuroaxonal degeneration and disability (36). In line with these findings, gene expression studies performed with cortical lesions of MS patients have identified significantly increased expression levels of IgG-associated molecules along with DNA repair, axonal transport, oxidative stress, apoptosis and remyelination-related genes (38). Also, MS patients with progressive disease forms show trends towards exhibiting increased numbers of CNS lesions with IgG and complement deposits as compared to RRMS patients (39).

The structure and cellular contents of TLOs are being increasingly understood by ongoing research. TLOs are mainly composed of antibody producing plasma cells and Th17-type CD4⁺ cells and thus resemble other lymphoid organs of the body. Follicular dendritic cells that are of vital importance for affinity maturation and production of high affinity antibodies are also abundantly found in TLOs. There is evidence suggesting that Th cells play a particularly important role in TLO formation by releasing B cell attracting chemokines. Notably in EAE induced by immunization or passive transfer of MS patients' T cells, TLOs may also occur in the CNS of experimental animals (36).

Antibodies and the Complement System In Disability Progression

Increasingly recognized significance of B cells in MS has brought forward antibodies, major products of mature B cells, as the main culprit of B cell-inflicted brain damage. CSF-specific oligoclonal bands represent a collection of immunoglobulins that are believed to be indicative of intrathecal autoreactive B cell activity. Not surprisingly, IgM oligoclonal bands and increased CSF IgM index have been associated with disability progression in MS. Especially, myelin reactive IgM-type oligoclonal bands may predict long-term (as long as 10 years) disease progression. By contrast, IgG-type oligoclonal bands and MRZ reaction have not been found equally beneficial in prediction of long-term disease progression (20).

Research of the last decade has identified a plethora of antibodies in MS patients. In the context of disability progression, antibodies directed against glial cell surface molecules (e.g. neurofascin-155) and axonal antigens (e.g. neurofilament light chain and contactin-1) appear to be principally associated with disability in MS (40). Serum levels of neurofilament light chain antibodies are significantly higher in SPMS and primary progressive MS (PPMS) patients (40). Moreover, primary neuronal cells incubated with these antibodies show reduced viability and myelin oligodendrocyte glycoprotein (MOG)-immunized EAE animals treated with these antibodies display enhanced disease severity and prevalence indicating involvement of neurofilament light chain antibodies in neuroaxonal degeneration and/or CNS inflammation (41).

Neurofascin-155 and contactin-1 are adhesion molecules involved in attachment of paranodal axolemmal membrane and myelin sheath. These antibodies are mainly found in sera of chronic inflammatory demyelinating polyneuropathy (CIDP) patients and lead to reduced nerve conduction through a specific mechanism called nodopathy (42). These two antibodies have also been identified in MS patients and administration of these antibodies into EAE animals has been shown to induce complement deposition and neuronal damage in the CNS. Notably, neurofascin-155 and contactin-1 antibodies are more prevalent in progressive forms of MS and are associated with increased EDSS scores (43).

The role of the complement system in demyelination has been largely disregarded and until recently attracted little attention. As the disability increases in MS, the number of CNS lesions with complement factor deposits tend to increase. Interestingly, among these complement factors, the classical complement pathway factor C1q, which is primarily activated by circulating antibodies, is abundantly found in MS lesions and often co-localize with IgG deposits, myelin proteins and macrophages (39). These findings suggest that complement factors accumulate on myelin sheaths upon activation with antibodies leading to membrane attack complex formation and subsequent myelin destruction. Presumably, complement breakdown products with chemoattractant features might also activate macrophages and lead to release of neurotoxic mediators. In line with these findings, CSF levels of complement factor C3 have been found to be correlated with neurofilament light chain levels and EDSS scores of MS patients. While higher CSF C3 levels are associated with increased disability in MS, no such association has been demonstrated in other neurological disorders characterized with CNS inflammation (44).

T Cell Mediated Immunity and Cytokines

With regard to association with disability, especially ELISA-based serum cytokine measurements may show diverse and sometimes opposite results. Cell-specific cytokine production studies based on measurement of intracellular cytokine levels with flow-cytometry or detection of supernatant cytokine levels of cultured and antigen stimulated peripheral blood mononuclear cells (PBMC) may yield more consistent and reproducible results.

Serum and/or PBMC levels of TNF- α , IL-1b, CXCL1 and CXCL5 have been found to be correlated with EDSS scores of MS patients (45, 46). Exact underlying mechanisms of these associations have not been studied. While TNF- α and IL-1b are involved in microglia-mediated oxidative stress and neuronal apoptosis (45), TNF- α , CXCL1 and CXCL5 are potent neutrophil chemotactic factors (46). Therefore, these associations emphasize the significance of innate immunity in disability progression in MS.

Lower levels of Th2 and regulatory T cell (Treg) cytokines IL-4 and IL-10 have been associated with higher EDSS scores in MS. Likewise, reduced peripheral blood Treg prevalence is associated with an increased EDSS score. These findings are congruent with the notion that Th2 and Treg cells have MS preventing action (47, 48) and thus individuals with reduced Th2 and/or Treg activity are more likely to develop a more severe disease course. Although IL-9, produced by Th17 and Th9 cells, is generally known to promote MS pathogenesis, MS patients with higher dendritic cell and CSF IL-9 levels have been shown to display lower EDSS scores (49). Immunological mechanisms underlying this association needs to be further studied.

Although Th1 and Th17-type cytokines play a major role in MS pathogenesis, their impact on disability progression is somewhat controversial. Higher levels of IFN- γ producing CD8⁺ cells and IL-17 producing CD4⁺ cells have been associated with worse disease outcome. In most studies, no correlation has been found between EDSS scores and intracellular or serum levels of IL-17 and IL-23. In a notable study, IL-17 and IL-22 levels have been found to be correlated with CNS lesion numbers but not EDSS scores of MS patients (45, 47, 48, 50). As to EDSS alterations under disease modifying drug treatment, serum levels of cytokines have not proven to be reliable predictive biomarkers.

Conclusive Remarks

Among several immunological factors, innate immunity related mediators and certain antibodies stick out as predictors of disease progression in MS. Recent research studies have underscored the significance of axoglial antibodies in disability progression of MS and exact mechanisms by which these antibodies exert CNS damage need to be further scrutinized. Better understanding of the interplay between microglial activity, oxidative stress and neuroaxonal degeneration may pave the way to development of novel therapeutics that are effective in progressive stages of MS.

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