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Emerging role of neuregulin-1beta1 in pathogenesis and progression of multiple sclerosis

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Multiple sclerosis (MS) is a chronic immune-mediated disorder of the central nervous system (CNS) that causes focal demyelinating lesions, followed by axonal and neuronal degeneration. Several genetic and environmental factors are found to be associated with MS incidence. While MS etiology seems to be multifactorial and needs further elucidation, it is understood that the response of an immune system to specific myelin antigens triggers the onset of MS (Dendrou et al., 2015). However, how the autoimmune response initiates against myelin, and the cellular and molecular mechanisms underpinning the development and progression of MS are not fully understood. Thus, deconstructing MS pathogenesis is of paramount importance for identifying novel diagnostic and therapeutic targets for this complex disease.

Different components of the adaptive and innate immune responses are implicated in the pathogenesis of MS (Dendrou et al., 2015). An adaptive immune response is traditionally known to play a major role in the initiation of demyelination in MS and in its animal model, experimental autoimmune encephalomyelitis (EAE). Autoreactive T cells of both helper T cell 1 (Th1) and Th17 subsets, along with $CD8^+$ cytotoxic T cells are among the main effector cells in this process (van Langelaar et al., 2020). Growing evidence suggests that regulatory T cells (Tregs) also play a crucial modulatory role in the autoimmune response in the EAE mice. Tregs are involved in the resolution of neuroinflammation and recovery in EAE through their anti-inflammatory properties. Recent studies on the motility differences in Tregs and effector Th17 cells have proposed three putative mechanisms for an immunomodulatory role of Tregs in MS that include reducing the number of antigen-presenting cells, limiting the access of Th17 to these cells, and inhibiting calcium signaling in Th17 cells (Othy et al., 2020). Autoreactive T cells are also known to cause neuropathologic effects through their interactions with the cells of the innate immune responses (Dong and Yong, 2019). Therefore, in recent years, there has been increasing emphasis on the role of peripheral and CNS innate immunity in the pathogenesis of MS and EAE.

Infiltrating monocyte-derived macrophages (MDMs) and resident microglia are two important members of the innate immune response in the CNS of EAE animals. Microglia and MDMs demonstrate diverse phenotypes in EAE and are polarized along a spectrum of pro-inflammatory/neurodegenerative to anti-inflammatory, neuroprotective/pro-regenerative phenotype, based on the stage of the disease (Wang et al., 2019). Since microglia and MDMs are not readily distinguishable in the CNS tissue morphologically or immunocytologically, understanding their specific functional roles in regulating MS pathogenesis and recovery has been historically challenging. However, with recent technical advances, new evidence shows distinct gene expression as well as temporal and spatial modulatory roles for microglia and MDMs in EAE mice or in response to the same inflammatory stimuli. Studies by Yamasaki and colleagues demonstrate that MDMs are predominantly implicated in the initiation of demyelination at the onset of EAE, while microglia are mainly involved in myelin clearance (Yamasaki et al., 2014). Intriguingly, subsequent fate mapping and single-cell RNA sequencing of microglia by Plemel and colleagues unraveled distinct subpopulations of microglia with diverse activation states, and suggested a complicated cross-talk between microglia and MDMs in demyelinating lesions that is critical to prevent MDMs from invading the CNS tissue (Plemel et al., 2020). These studies, among others, have collectively pointed to the critical role of the innate immune response in modulating both the injury and repair processes in MS. However, the endogenous mechanisms that modulate the neuro-degenerative or neuro-regenerative nature of the immune responses during the pathologic events of MS are not fully understood.

In efforts to uncover the endogenous mechanisms that regulate the phenotype of innate and adaptive immune responses in MS, our group has recently uncovered an important immunomodulatory role for Neuregulin-1β1 (Nrg-1β1) in MS pathogenesis (Kataria et al., 2021; **Figure 1**). This article provides a perspective on these new findings. The Neuregulins belong to a family of signaling proteins with diverse regulatory roles in development and physiology of neurons, axons, myelin, and glial cells in the CNS. Nrg-1β1 has been emerged as a mediator of CNS injury and repair in recent years (Kataria et al., 2019). Nrg-1β1 is mainly expressed by neurons and cells of the oligodendrocyte lineage, while its receptors, ErbB-2, ErbB-3, ErbB-4, are expressed widely by various cell types in the CNS (Kataria et al., 2019). Our studies in the EAE mice showed that Nrg-1β1 protein is down-regulated in the spinal cord, and peripherally in the blood and spleen of these mice (Kataria et al., 2021). Nrg-1β1 down-regulation preceded the disease onset as its levels were significantly reduced in the pre-symptomatic phase of EAE that persisted after the peak of the disease. Previously, we also found significantly dysregulated levels of Nrg-1β1 protein in demyelinating lesions of spinal cord injury and lysolecithin-induced focal demyelination model (Alizadeh et al., 2018; Kataria et al., 2018), suggesting its broader impact on CNS demyelination. Nrg-1β1 dysregulation had functional ramifications on the onset and progression of EAE as restoring its levels with peptide treatment using recombinant human Nrg-1β1 was sufficient to attenuate disease severity and progression in EAE mice. Nrg-1 treatment delivered at pre-symptomatic, onset, peak, and post-peak of the EAE was effective in improving disease outcomes. Pre-symptomatic administration of Nrg-1 delayed the onset of symptoms in EAE mice, and when appeared, the symptoms were less severe. These findings suggest dysregulation of Nrg-1 is an early pathology in EAE and its promise as a treatment with a wide therapeutic window of opportunity for MS.

Relevance of preclinical findings in animal models to human MS pathology is of paramount importance to validate the translational impact of these discoveries for diagnosis and therapeutic development. Assessment of MS brain confirmed down-regulation of Nrg-1 in active demyelinating plaques as compared to normal-appearing white matter in the same patients. Importantly, Nrg1-β1 protein levels were declined in the plasma of individuals with clinically isolated syndrome (CIS). CIS individuals present with the first episode of neurological impairment characterized by inflammation or demyelination on magnetic resonance imaging consistent with an MS relapse. These individuals have a high probability of transition to relapsing-remitting MS in a few years. Analysis of the plasma levels of Nrg-1 in CIS and healthy control individuals showed a significant reduction in Nrg-1β1 plasma levels of CIS individuals who eventually developed relapsing-remitting MS. These findings suggest that dysregulation of Nrg-1β1 in the early stages of MS pathogenesis may present a potential disease biomarker, as well as a promising therapeutic target for MS. This opens a new direction for future research with a larger number of human samples of all MS subtypes to verify the association between Nrg-1β1 levels and disease onset and progression both peripherally and within the CNS.

From the mechanistic point of view, these studies unraveled that increasing the availability of Nrg-1β1 can modulate both the innate and adaptive immune responses in the spinal cord of EAE mice (Kataria et al., 2021; **Figure 1**). Interestingly, Nrg-1β1 treatment did not affect the overall number of $CD3^+/CD4^+$ T cells in the blood or the spinal cord of EAE mice, while it significantly changed the phenotype of T cell population in the EAE lesion. Availability of Nrg-1β1 reduced the number of effector $CD4^+$/interferon γ (IFNγ) expressing Th1 cells while increasing anti-inflammatory Tregs. This modulatory effect was associated with a concomitant reduction in the spinal cord levels of Th1 related cytokines including IFNγ, interleukin 2 (IL-2), and IL-16. Intriguingly, this work demonstrated that Th1 regulatory action of Nrg-1β1 treatment happens within the CNS lesions and not in the periphery, although dysregulation of Nrg-1β1 occurs both peripherally and within the CNS during disease pathogenesis. To decipher further information concerning the cellular source of Nrg-1β1 effects and peripheral verses CNS mediated mechanisms of Nrg-1β1 in pathogenesis of EAE, passive transfer studies in EAE mice and CNS specific deletion of Nrg-1β1 is warranted.

Therapeutic restoration of Nrg-1β1 exert a more pronounced effect on monocyte infiltration in EAE mice resulting in a robust decrease in MDMs in the spinal cord lesions with no effects on the recruitment of resident microglia to EAE lesions (Kataria et al., 2021; **Figure 1**). Interestingly, this phenomenon was associated with reduced levels of chondroitin sulfate proteoglycans (CSPGs) and matrix metalloproteinase 9 (MMP9) in EAE lesions. Of note, MMP9 and CSPGs play critical roles in the integrity of the blood-CNS-barrier and leukocyte trafficking in MS and EAE. In the perivascular cuff, CSPGs are known to aid accumulation of leukocytes and their entry to the CNS parenchyma of EAE and MS (Stephenson et al., 2018). MMP9 also has an established role in pathogenesis of MS by increasing permeability of blood-CNS-barrier and myelin damage. Hence, the ability of Nrg-1β1 in attenuating CSPG and MMP9 production is an important therapeutic effect. Overall, these findings suggest that Nrg-1β1 potentially influences the recruitment of MDM in EAE lesions by regulating the permeability of the blood-CNS-barrier. Notably, MMP9 and CSPGs are primarily produced by pro-inflammatory leukocytes, microglia and astrocytes in EAE. Thus, downregulation of MMP9 and CSPGs by Nrg-1β1 treatment in EAE may reflect the immunomodulatory role of Nrg-1β1 in supporting the phenotype shift of both MDMs and microglia from pro-inflammatory M1 to neuroprotective M2 (Kataria et al., 2021). This phenotype shift in microglia and MDMs by Nrg-1β1 treatment was also reported previously by our group in rat spinal cord injury lesions (Alizadeh et al., 2018). Key pro-inflammatory cytokines associated with the M1 phenotype of microglia and MDMs in MS and EAE include IL-1β, IL-6, and tumor necrosis factor α, and these cells are also known to produce higher levels of reactive oxygen species (Wang et al., 2019). The phenotype shift in microglia and MDMs under Nrg-1 treatment was also accompanied by a decrease in the spinal cord levels of IL-1β, IL-6, tumor necrosis factor α and reactive oxygen species in EAE (Kataria et al., 2021).

Pro-regenerative M2 macrophages and microglia play a supportive role in remyelination through the secretion of mediators that promote oligodendrocyte progenitor cell differentiation and maturation (Wang et al., 2019). In our previous work using lysolecithin-induced focal demyelination model, we showed Nrg-1β1 can promote the generation of mature oligodendrocytes that subsequently accelerated and enhanced remyelination in demyelinating lesions of the spinal cord (Kataria et al., 2018; **Figure 1**). These results along with the observations from the recent study propose that Nrg-1β1 may promote oligodendrocyte maturation through modulation of microglia and MDMs along the spectrum of pro-inflammatory to anti-inflammatory, pro-regenerative phenotype. Altogether, these findings have provided new insights into the putative mechanisms by which

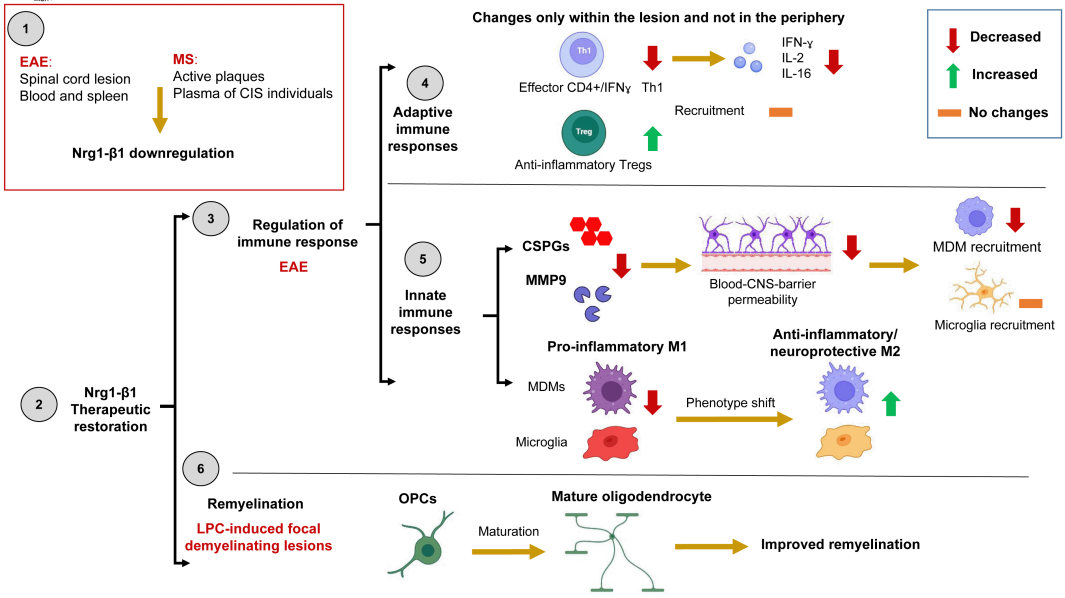

Figure 1 | **The current proposed role and mechanisms of Nrg-1β1 in pathophysiology of MS and EAE.**
(1) We have demonstrated that expression level of Nrg-1β1 protein is dysregulated in the spinal cord lesions, peripheral blood, and spleen of EAE mice, as well as active plaques of individuals with MS and plasma of CIS individuals. (2) Restoration of Nrg-1β1 levels using recombinant human Nrg-1β1 peptide therapy has been beneficial for recovery in animal models of MS through various mechanisms: (3) Modulation of the immune responses through the regulation of both adaptive and innate components of neuroinflammation. (4) In adaptive immune response, while Nrg-1β1 treatment does not affect the total number of T helper cells in the blood or to the spinal cord of EAE, it significantly decreases the population of $CD4^+/IFN\gamma^+$ Th1 cells that leads to reduced levels of inflammatory cytokines such as IFNγ, IL-2 and IL-16. Nrg-1β1 treatment increases anti-inflammatory Tregs within the EAE lesions in the spinal cord. (5) In innate immune response, restoring Nrg-1β1 levels reduces monocytes in the blood and their infiltration into the CNS and consequently a decrease in the number of MDMs, while the recruitment of microglia remains unaffected. This was correlated with a reduction in the production of CSPGs and MMPs that are known mediators involved in the blood-CNS permeability in EAE and MS. Availability of Nrg-1β1 also supports a phenotype shift from pro-inflammatory M1 to neuroprotective M2 in both microglia and MDM populations. (6) Using LPC model, we have demonstrated that Nrg-1β1 treatment promotes differentiation of OPCs to mature myelinating oligodendrocytes that results in accelerated and improved remyelination of demyelinating lesions of the spinal cord. CIS: Clinically isolated syndrome; CNS: central nervous system; CSPGs: chondroitin sulfate proteoglycans; EAE: experimental autoimmune encephalomyelitis; IFNγ: interferon γ; IL: interleukin; LPC: lysolecithin induced focal demyelination; MDM: monocyte-derived macrophage; MMP9: matrix metalloproteinase-9; MS: multiple sclerosis; Nrg-1β1: neuregulin-1beta1; OPC: oligodendrocyte progenitor cell; Th1: helper T cell type 1; Tregs: regulatory T cells. This figure is created with BioRender.com.

Nrg-1β1 dysregulation regulates responses in EAE. The current data propose that Nrg-1β1 can modulate both the innate and adaptive immune responses at the CNS levels, and its effects peripherally are mainly through the regulation of monocytes and their infiltration into the CNS. This, however, raises the possibility that Nrg-1β1 regulation of T cell responses in the CNS lesions of EAE might be indirectly through modulation of microglia and MDMs phenotype and function, which provides new directions for further investigations.

In conclusion, understanding the underlying endogenous mechanisms that drive MS pathogenesis and progression is pivotal to deconstruct disease complexity, and unravel specific targets for MS diagnosis and treatment. Our new findings highlighted in Kataria et al. (2021) have proposed Nrg-1β1 dysregulation as a disease mechanism implicated in the early phase of MS pathogenesis and a potential predictive marker for disease progression. Importantly, the functional relevance of Nrg-1β1 dysregulation was confirmed by its therapeutic potential to ameliorate disease severity and progression in EAE mice. Mechanistically, the availability of Nrg-1β1 appears to play a role in immune homeostasis in the CNS as its dysregulation is associated with "monocyte extravasation" and promoting T helper 1 effector cell response in EAE. Further investigation using animal models with Nrg-1 genetic deletion is warranted to verify whether absence of Nrg-1 could trigger immune dysregulation peripherally and/or at the CNS. Moreover, to establish Nrg-1β1 as a potential disease marker for MS with both diagnostic and prognostic values in clinical settings, further investigation of its plasma levels in a larger size of MS patient samples is required.

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