The Role of Sex Hormones in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelination disorder with an immune-mediated pathophysiology that affects the central nervous system (CNS). Like other autoimmune conditions, it has a predilection for female gender. This suggests a gender bias and a possible hormonal association. Inflammation and demyelination are hallmarks of MS. Oligodendrocytes are the myelinating cells of the CNS and these continue to be generated by oligodendrocyte precursor cells (OPCs). The process of remyelination represents a major form of plasticity in the developing adult CNS. Remyelination does occur in MS, but the process is largely inadequate and/or incomplete. Current treatment strategies primarily focus on reducing inflammation or immunosuppression, but there is a need for more extensive research on re-myelination as a possible mechanism of treatment. Previous studies have shown that pregnancy leads to an increase in OPC proliferation, oligodendrocyte generation and the number of myelinated axons in the maternal CNS. Studies have also suggested that this remyelination is possibly medi-

ated by estradiol. Sex hormones in particular have been shown to have an immuno-protective effect in TH1-driven autoimmune diseases. The aim of our article is to review the available research on sex hormone-specific immune modulatory effects, assess its remyelination potential in MS, and suggest a future path for more extensive research on sex hormone as a target for therapeutics in MS.

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Introduction

Multiple sclerosis (MS) is one of the leading causes of disability in young patients, and it is more common in women. The etiology remains unknown but may involve abnormalities in T lymphocyte responses [1]. CD4 and CD8 are subsets of T lymphocytes. CD4 (helper T cells) are the most prolific cytokine producers, including pro-inflammatory Th1-type cytokines, which induce tissue damage and perpetuate autoimmune responses. The Th2-type cytokines have more of an anti-inflammatory response.

In 2005, a third T-cell subset, known as Th17, was identified and is characterized by the production of IL-17.

This may have evolved for host protection against microbes that are not addressed by Th1 or Th2 immunity [2, 3]. MS is characterized by inflammation and axonal damage, which lead to neuronal injury [4].

Females have a more robust immune system than males and the female preponderance in autoimmune diseases suggests a role for hormones. Vaccination response in females as well as the relative resistance to infections is eliminated after menopause. The immune senescence of menopause has been linked to the altered sex steroid status [5]. The involution in the thymus has been partially attributed to the increase in sex steroids at puberty. Ablation of sex steroids may trigger thymic regrowth and improved T-cell activity in the setting of hematopoietic stem cell transplantation [6].

It is now known that the major reproductive hormones like estradiol, progesterone, prolactin and glucocorticoids increase during pregnancy and are critical modulators of the immune function, inducing immune tolerance. This seems to be achieved by decreasing adhesion molecules, matrix metalloproteinases (MMP), shifting cytokines, decreasing antigen presentation and increasing the number of T-regulatory cells (Tregs). Treg cells are a subset of T lymphocytes that primarily function to modulate and suppress the immune system and reduce pathological self-reactivity as seen in MS and other autoimmune conditions. All these changes decrease inflammation and benefit patients with autoimmune Th1-driven disorders [7].

Pregnancy is associated with a strong Th2 response in order to reduce miscarriage. It is considered an immunotolerant state, where the maternal immune system needs to adapt to fetal allogeneic tissue [7]. Mother and fetal tissue have a bidirectional exchange of cells; fetal tissues are considered semiallogeneic, since it has antigens of paternal and maternal origin. This intimate communication leads to microchimerism and to a state of transient T-cell tolerance specific for paternal antigens [8].

Symptoms and disease activity of several cell-mediated Th1/Th17 autoimmune diseases decline during pregnancy and exacerbate after delivery. This has been well documented for MS, in which the relapse rate significantly decreases during pregnancy and this has been linked to rise in hormones such as estradiol. The Pregnancy in MS study is a large prospective analysis, which included 254 women and 269 pregnancies. Patients were followed for at least 12 months postpartum, the relapse rate fell 70% during the third trimester. Interestingly, during the first 3 months postpartum, the relapse rate increased by 70% [9].

The immune response may be gender specific and dimorphic in nature [10]. The dimorphism may be based on gender differences in sex steroids that influence both humoral and cell-mediated immunity [11]. Sex-hormone receptors are present on regulatory T cells [12]. Animal models have shown that autoimmunity is promoted by self-antigen expression in medullary thymic epithelial cells. The developing T cells that recognize these self-antigens within the thymus undergo clonal deletion. Androgens upregulate autoimmune regulator-mediated thymic tolerance to protect against autoimmunity [13]. Testosterone, perhaps through lipid mechanisms, may exert an immunosuppressive effect [14]. Treating obese strain chickens with testosterone or testosterone analogs provided significant protection against spontaneous autoimmune thyroiditis [15].

Possible Mechanisms by Which Sex Steroids Could Impact Re-Myelination

Neurons and glia are endowed with high-affinity steroid receptors [16]. Astrocytes in the rim and center of demyelinating lesions appear to be the primary source of steroidogenic enzymes and receptor expression [17]. In a study of OKT8-positive cells, the restriction of estrogen receptors (ERs) to T cells bearing the “suppressor-cytotoxic” phenotype may offer a possible pathway for the modulation of T-cell suppressive activities by estrogens [18]. Sex steroids may also influence host defense by regulating the ability of macrophages to participate in immune responses [19]. Decreased concentrations of sex steroids are associated with higher serum levels of proinflammatory cytokines such as tumor necrosis factor- α and interferon- γ [20, 21].

Experimental autoimmune encephalomyelitis (EAE) in animals has many comparable pathologic features to MS [22]. In EAE, estrogen can activate macrophages and microglia, thereby increasing the induction of regulatory B cells. This regulatory feedback may be a component of neuroprotection [23]. The priming of B cells by estradiol is an important regulatory mechanism in neuromodulation [24]. Female sex steroids may shift T cells towards an expansion of Treg and Th2 cells; cytokines produced by Th2 cells generally suppress EAE [25, 26].

Microglial inflammation may be an early indicator of axonal injury, which contributes to neuronal dysfunction [27]. Activated microglia contributes to demyelination, perhaps due to altered production of nitric oxide and tumor necrosis factor- α . The latter can be toxic to cen-

tral nervous system (CNS) cells, including oligodendrocytes [28]. Hypoxia induced proinflammatory cytokines reduce phagocytosis in BV-2 microglia cells. These actions can be prevented by sex steroids through switching from a proinflammatory to an anti-inflammatory phenotype [29], by acting directly on the microglia as well as on astroglial regulation [30].

Other postulated pathways for demyelination in MS, include programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) [31]. PD-L1 is a programmed death ligand, which along with its receptor PD-1 has been postulated to inhibit T-cell proliferation and induce apoptosis of activated T cells, thereby suppressing T-cell immune response. There is evidence in EAE that PD-1 interaction with PD-L1 is critical for estrogen induced protection in EAE [32]. MMP play a key role in the influx of inflammatory cells into the CNS. In MS, MMP-9 levels are elevated and predict the onset of new active lesions on MRI imaging [33]. Estriol decreased MMP-9 levels along with a decrease in CNS infiltration by T-cells and monocytes. These effects are mediated through ER α [33].

FDA-approved medications for MS reduce inflammation and relapse rate but have only a modest effect on gray matter atrophy and disability [34]. Current agents do not promote re-myelination [35]. Recently, there has been interest in the modulatory role of sex steroids and their effect on re-myelination. Sex steroids may achieve remyelination through several mechanisms, such as interactions with astroglia, insulin-like growth factor-1, and recruitment of oligodendrocytes [16]. Sex steroids may modulate chemokine expression and signaling, relevant in ischemic and demyelinating diseases [36].

Previous studies have shown that estriol has been associated with a decrease in the delayed type hypersensitivity response to a recall antigen, a decrease in IF- γ (interferon gamma) message and in gadolinium-enhancing lesions on brain MRI [37]. Reducing inflammation could also be a way in which estriol indirectly promotes remyelination. During pregnancy, oligodendrocyte precursor cells proliferate leading to an increase in the number of myelinated axons [38]. The role of estriol and other sex hormones in regards to remyelination is still to be determined.

Estradiol, Synthetic Estrogens and Estriol

Estrogens bind to 2 known ER, ER α , and ER β , in EAE. ER α -selective ligands may have implications in the development of therapeutic strategies for MS [39]. In contrast,

treatment with selective ER modulators (SERMs) indicated that ER β was capable of providing protection against neurodegeneration, whereas ER α was anti-inflammatory [40, 41]. ER β ligands have been proposed as the next-generation estriol treatment, given their protective effect across both sexes [42]. It should be noted that the immunomodulatory effects of tamoxifen may be independent of the estrogen-receptor with its immunomodulation mediated through the multidrug resistance gene product, permeability-glycoprotein [43].

The beneficial effect of pregnancy and the influence of sex hormones with the immune system have also been observed in the EAE mouse model, most noticeable in the third trimester of pregnancy. This effect may be mediated at least in part by estriol, which rises during the third trimester of pregnancy and then falls precipitously postpartum when EAE activity increases. Endogenous estriol reflects the pattern of disease activity in the EAE model in relation to MS and pregnancy. When administered to mice prior to inoculation of EAE, exogenous estriol reduces disease activity [44]. Female sex steroids may facilitate immune cell recruitment into the CNS via glia cells, thus modulating cell-cell communication. This can reduce neurodegeneration in MS [45].

In the brain of aging female rats, increased neurolipofuscin and decreased antioxidant enzymes are seen. Treatment with Estradiol reversed these changes to near normal levels [46]. Estrogen may differentially regulate metabolic enzymes and cholinesterases in the brain leading to differential remodeling of neuronal circuitry [47]. Estrogen can restore resistance to Theiler's virus-induced demyelinating disease by induction of immune responses, infiltration of active immune cells into neural tissue and by the inhibition of NF κ B function [48].

Treatment with synthetic estrogens in the EAE model in Lewis rats ameliorates the clinical and histological manifestations [49]. Estradiol supplementation in gonadectomized groups increased the number of oligodendrocytes and promoted myelination and axon conduction in the corpus callosum [50]. Estradiol binds to specific intracellular receptors acting at the genomic level. However, estrogens may be neuroprotective through both genomic and non-genomic pathways. The intrinsic antioxidant properties of estrogen may provide a chemical shield for neurons [51].

However, not all studies have confirmed a beneficial relationship between estradiol and demyelination. In men with MS, there was a positive correlation between estradiol concentrations and brain damage [52]. This raises the existence of possible differences among the estrogens.

The role of Estriol in MS has been recently reviewed [53]. Estriol has been used more extensively than estradiol via transvaginal route. The potential benefits of this transvaginal route include limited systemic exposure and lack of a second pass effect seen with oral estriol. Moreover, estriol is a weaker estrogen than 17-Beta estradiol and as such may be able to be used in males. In EAE, both females and males had decreased proinflammatory cytokine profile with estriol treatment [54]. Estriol can generate tolerogenic dendritic cells that are resistant to the inflammatory response seen in EAE [55]. Estriol also prevented hippocampal changes seen in EAE [37]. It has also been shown to inhibit nuclear transcription factor kappa B, which controls many immune functions including T-cell function [56]. In elegant studies by Tiwari-Woodruff and Voskuhl [41], oral estriol has been shown to have a positive effect on gadolinium, thereby enhancing brain lesion in MS [54]. The aim was to simulate physiologic estriol levels in pregnancy with 8 mg of oral estriol. However, this oral route has a significant first-pass effect potentially increasing inflammatory markers and hypercoagulability [57]. Moreover, oral estriol may also have a second-pass effect. The potential increase in hepatic sex hormone binding globulin with oral administration can lower testosterone levels compared to the transdermal or transvaginal route. Thus, bypassing hepatic metabolism will likely create more stable levels of estrogens and more studies using this approach are needed.

Progesterone and Progestins

Progesterone is believed to have neuroprotective, promyelinating, and anti-inflammatory effects in the nervous system [58]. In EAE mice, progesterone attenuates the clinical severity, decreases demyelination and neuronal dysfunction, increases axonal counts, reduces the formation of amyloid precursor protein profiles, and decreases the aberrant expression of growth-associated proteins. The progestin Nesterone protects against hippocampal abnormalities and improves functional outcomes in mice with chronic EAE [59]. In contrast, Babri et al. [60] reported that intrahippocampal injection of progesterone alone or in combination with vitamin C had no improving effects on memory. Progesterone and Nestorone also decrease neuroinflammatory responses, specifically astrocyte and microglial cell activation [61]. Therefore, some progestogens are promising therapeutic candidates for promoting the regeneration of myelin.

Progesterone may work through the mitochondrial translocator protein, which is the rate limiting step in the conversion of cholesterol to pregnenolone and its derivatives [62]. The mitochondrial translocator protein is up-regulated in microglia and astroglia during neural inflammation. Etofoxine, an anxiolytic drug, which is also a mitochondrial translocator protein ligand attenuated the severity and improved recovery in EAE. Its use was associated with less peripheral immune cell infiltration of the spinal cord and increased oligodendroglial regeneration [62]. Pre-treatment with progesterone before induction of EAE had a protective effect on mitochondrial morphology, fission/fusion protein mRNA with inhibition of microgliosis [63]. This allows the recovery of neurosteroidogenesis [64]. The re-myelinating effect of progesterone is dependent on the progesterone receptor [61].

In the EAE model of MS, progesterone reduced the severity of the disease along with reduced demyelination and inflammatory response [65]. Progesterone also exerts protective effects on axonal pathology in mice with EAE [66]. In addition to reducing disease severity, neuronal dysfunction, demyelination in EAE, progesterone also reduces the formation of amyloid precursor proteins and growth-associated proteins [67].

Testosterone

Serum testosterone was significantly lower in women with MS than in controls [52]. Low testosterone has been reported in 40% of men with MS, correlating with physical, cognitive disability [68] and worse clinical outcomes [69]. In 10 men with relapsing remitting MS (RRMS), testosterone gel improved cognition, lean body mass and reduced brain atrophy [70]. No effect on contrast enhanced brain lesions in either number or volume was observed. In another small study of 10 patients, testosterone treatment significantly reduced delayed type hypersensitivity, and decreasing CD4+ T cell percentage and increasing NK (Natural Killer) cells [71]. The authors concluded potential neuroprotective effect based on the increase in brain-derived neurotrophic factor and platelet-derived growth factors. Testosterone therapy for 12 months in patients with RRMS was associated with a significant increase in gray matter in the frontal cortex. Testosterone treatment has the potential to perhaps reverse or halt neurodegenerative changes associated with MS [34]. Over 50% of MS patients have cognitive deficits with impaired hippocampal-dependent memory impairment in over

30% of these patients [72]. In EAE, testosterone administration restores excitatory synaptic transmission in the hippocampus [72]. Also, in EAE models, there seems to be a protective role of testosterone. EAE is less severe in female mice pretreated with dihydrotestosterone, and is more severe in castrated mice. This can be ameliorated with testosterone replacement [44]. Testosterone has the potential to be a less toxic neuroprotective agent in RRMS [68]. It has the added benefit of possibly improving bone mineral density [73].

Sex Steroid Combinations

Combining estrogen with progesterone in EAEs inhibited the development of major neurochemical abnormalities and clinical signs of the disease [74]. Neuroprotective, promyelinating and immunosuppressive mechanisms may be responsible for these benefits. Estradiol and progesterone may reduce central immune responses in the brain and regulate local growth factors, oligodendrocyte, and astrocyte function [75]. Interestingly, individuals with a high ratio of estrogen to progesterone ratio had a significantly greater number of active MRI lesions than those with a low ratio [76]. There is a paucity of data using sex steroid combinations in demyelinating disease.

Conclusion

Systemic glucocorticoids in MS play a role in treating acute exacerbations but do not influence long-term outcomes. Vitamin D, corticosteroids, and sex steroids are all derived from cholesterol metabolites and are ligands for nuclear receptors [77]. Activation of these receptors results in the regulation of multiple gene transcription involved in general homeostatic and adaptation networks, including immunomodulation. Distinct ligands affect the function of both myeloid and lymphoid cells and can promote a less proinflammatory state. A functional synergy for the immunomodulatory effects of vitamin D3 and 17- β estradiol offers new options for RRMS patients [78]. It remains to be determined which of the 3 estrogens are optimal. There is some evidence that for immunoregulation, estriol may offer greater benefits than estradiol [79]. There are very few studies using Estrone in demyelinating diseases [38]. The pharmacokinetic profile of fetal estrogen estetrol may lend itself to once daily administration [80] and remains to be studied in MS.

Role of Sex Hormones in Remyelination

Studies examining sex steroids in demyelination are promising. Many mechanisms remain undefined or even unidentified. Estrogen prevents changes in gut microbiota associated with EAE [81]. Such mechanisms may include the promotion of specific gut microbiota, and the cross talk between the gut microbiota and sex hormones may help immune-regulation resulting in neuroprotection. Moreover, gender-specific responses to inflammation within the CNS are different and require more study. In a primary culture of human microglia and astrocytes, lesions in males with MS had upregulation of aromatase and increased TNF, ER α , and estrogen synthesis. In the lesions in females, 3 β -hydroxysteroid-dehydrogenase, a precursor of progesterone and the progesterone receptor is activated [17]. Smaller studies with testosterone have demonstrated a neuroprotective effect possibly from an increase in brain-derived neurotrophic factor and platelet-derived growth factors. It has also been associated with significant increase in gray matter in the frontal cortex in patients with RRMS, and has a potential to reverse or halt neurodegenerative changes associated with MS.

There is a great need in MS for therapeutic agents that promote re-myelination. The possibility that sex steroids either alone or in combination with other agents can induce re-myelination remains a main goal in managing patients with MS and warrants further research efforts.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

Mirla Avila MD has participated in advisory meeting and speaker engagements for patient education with Teva, Genzyme and Biogen. The rest of the authors have no conflicts of interest to declare. We have received no funding for this research.

Author Contributions

M.A. drafted the body of the manuscript. A.B. wrote the abstract, completed the formatting and other miscellaneous corrections. J.C. provided with expert insight, critical review of manuscript. A.N.P. also co-drafted the body of the manuscript.

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