Estrogen and Testosterone Therapies in Multiple Sclerosis

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Abstract

It has been known for decades that females are more susceptible to inflammatory autoimmune diseases including multiple sclerosis (MS), rheumatoid arthritis, and psoriasis. In addition, female patients with these diseases experience clinical improvements during pregnancy with a temporary ‘rebound’ exacerbation post partum. These clinical observations suggest an effect of sex hormones on disease suggest potential use of the male hormone testosterone and the pregnancy hormone estriol, respectively, for treatment of MS. A growing number of studies using the MS animal model experimental autoimmune encephalomyelitis (EAE) support a therapeutic effect of these hormones. Both testosterone and estriol have been found to induce anti-inflammatory as well as neuroprotective effects. Findings from two recent pilot studies of transdermal testosterone in male MS patients and oral estriol in female MS patients support the therapeutic potential of these hormones. In this paper, we review the pre-clinical and clinical evidence for sex hormone treatments in MS and discuss potential mechanisms of action.

1.Introduction

1.1. Inflammation vs neurodegeneration in multiple sclerosis

Multiple sclerosis (MS) is a heterogeneous inflammatory, demyelinating and degenerative disease of a presumed Th1-autoimmune origin that occurs in genetically susceptible individuals (Hemmer et al., 2002). The exact pathogenetic mechanisms are unknown but peripheral activation of autoreactive CD4+ T cells targeting proteins of the myelin sheath of neurons has been hypothesized as a key process in the development of the disease (McFarland and Martin, 2007). Upon activation, these cells cross the blood brain barrier to enter the central nervous system (CNS), recognize myelin antigens and initiate a chronic inflammatory cascade that results in demyelination of axons, mainly by macrophages (Sospedra and Martin, 2005). Involvement of humoral (antibodies and complement) and cellular mechanisms, as well as primary oligodendroglial degeneration and apoptosis have also been proposed (Lassmann et al., 2001). The pathological hallmark is the demyelinated plaque, which consists of well-demarcated areas characterized by loss of myelin and formation of astrocytic scars. However, it is becoming increasingly clear that axonal loss may be the major determinant for long-term, permanent disability. It is unclear whether all neurodegeneration is directly related to acute inflammation, since diffuse axonal damage may occur separately from pathological lesions (Evangelou et al., 2000) and even robust and effective immunosuppression with chemotherapeutic agents is not sufficient to stop accumulation of disability, in particular during later disease stages (Coles et al., 2006). Thus it appears that MS has both an inflammatory and a neurodegenerative component in its pathogenesis. Over the last decade, abundant neuroimaging and neuropathological studies have indicated a significant neurodegenerative
process in MS. Neuroimaging has demonstrated atrophy (Brex et al., 2000; Filippi et al., 2003; Ge et al., 2000; Losseff et al., 1996; Rudick et al., 1999; Stevenson et al., 1998), particularly in gray matter (Bakshi et al., 2001; Catalaa et al., 1999; Rudick et al., 1999). This gray matter atrophy has been shown to correlate better with permanent disability than does the white matter inflammatory marker of gadolinium enhancing lesions (Ge et al., 2000; Rudick et al., 1999; Stevenson et al., 1998). Also, abnormalities beyond classic white matter T2 hyperintensities, within “normal appearing white matter” (NAWM), have been shown using magnetization transfer, spectroscopy and diffusion weighted imaging (Catalaa et al., 2000; De Stefano et al., 1999; Filippi et al., 2000a; Filippi et al., 2000b; Gasperini et al., 1996; Narayanan et al., 1997; Santos et al., 2002; Tortorella et al., 2000). Furthermore, the degree of change in the NAWM may be a predictor of future clinical progression (Santos et al., 2002). Pathological findings in MS have described cortical lesions that were characterized by transected neurites (both axons and dendrites) and apoptosis with very little T and B cell infiltration (Bo et al., 2003; Peterson et al., 2001). Axonal transection has also been described within white matter lesions raising the possibility of Wallerian degeneration in white matter tracts.

In light of these observations, there is now a consensus by MS investigators that there is a need to discover novel treatment options, which combine neuroprotective properties with anti-inflammatory effects. In this paper, we will outline the scientific basis for sex hormones as putative treatment options in MS as well as other CNS diseases with both an inflammatory and a neurodegenerative component and review potential mechanisms of action.

1.2. Rationale for sex hormones as treatment options for MS

The concept that sex hormones may play a role in MS pathogenesis and disease activity and could therefore potentially be used for therapeutic interventions is based on two well-established clinical observations: a higher prevalence of MS in females compared to males and a decrease in disease activity during pregnancy, in particular in the third trimester. Below, we will briefly outline the evidence for these two phenomena and their relevance for sex hormone treatments in MS. For a comprehensive overview of this area we refer the reader to a recent review published elsewhere (Voskuhl, in press).

1.2.1. Gender gap—Many autoimmune diseases are more prevalent in women than in men. In MS, there is a female-to-male preponderance approaching 2:1 to 3:1 (Duquette et al., 1992) and recent evidence seems to suggest that the gender gap is widening (Orton et al., 2006). The causes for the sex bias in MS and other autoimmune diseases may include sex-linked genetic factors, sex differences in immune responsiveness, and/or sex steroid effects (Whitacre et al., 1999). Interestingly, a later onset of disease in male patients compared to female patients (Weinshenker, 1994) coincides with a decline in bioavailable testosterone in men (Swerdloff and Wang, 2004). Although only a minority of male patients with MS have demonstrated testosterone levels significantly below the normal range (Foster et al., 2003; Wei and Lightman, 1997), these findings suggest that testosterone may be protective in young men genetically susceptible to MS. There is an ongoing controversy whether or not established MS progresses at a different speed in men and women. A detailed review of the empirical evidence in this area can be found elsewhere (Voskuhl, in press). Taken together, the data suggest that men are less likely to develop clinical relapses and enhancing lesions on MRI but it remains unclear if there is a gender difference regarding progression of clinical disease or neurodegeneration on MRI. Generally, this is in line with a beneficial, anti-inflammatory effect of endogenous testosterone in MS.

1.2.2. The protective effects of pregnancy—It has been appreciated for decades that symptoms of patients with autoimmune diseases are affected by pregnancy and the post partum period. MS patients as well as individuals with other inflammatory autoimmune diseases such
as rheumatoid arthritis (RA) and psoriasis experience clinical improvement during pregnancy, with a temporary ‘rebound’ exacerbation postpartum (Abramsky, 1994; Birk et al., 1990; Confavreux et al., 1998; Da Silva and Spector, 1992; Damek and Shuster, 1997; Nelson et al., 1992; Runmarker and Andersen, 1995). The most definitive study of the effect of pregnancy on MS came in 1998 by the Pregnancy in Multiple Sclerosis (PRIMS) Group (Confavreux et al., 1998). This study followed 254 women with MS to one year post delivery and showed that relapse rates were significantly reduced from 0.7 per woman/year in the year before pregnancy to 0.2 during the third trimester. Rates then increased to 1.2 during the first 3 months postpartum before returning to pre-pregnancy rates. Together these data clearly demonstrated that late pregnancy is associated with a significant reduction in relapses, while there is a rebound increase in relapses postpartum. It is however unclear if this effect on relapse rate translates into a beneficial effect on long-term disability. One short-term 2 year follow-up study indicated that there is no ‘net’ effect of a single pregnancy on disability (Vukusic et al., 2004). However, a long-term study in 200 women showed that patients who had at least one pregnancy after onset were wheelchair dependent after 18.6 years, versus 12.5 years for the other women (Verdru et al., 1994), indicating a protective effect of pregnancy on long term disability accumulation. Thus, there is clear evidence that pregnancy has a potent short-term effect on inflammation and relapse rate but data regarding long term effects on disability are inconclusive.

Pregnancy is characterized by an array of biological changes that could mediate both immunomodulatory and neuroprotective effects. First, a pronounced systemic shift from Th1-type cellular immunity towards Th2-type humoral immunity can be observed during pregnancy (Whitacre et al., 1999). This immune shift, rather than a general immune suppression, is beneficial during pregnancy for two reasons: The fetus represents an ‘allograft’ in immunological terms, since it harbors antigens inherited from the father and the natural immunomodulation is thus important to prevent fetal rejection. On the other hand, the developing fetus depends on the mother for the passive transport of antibodies in light of its immature immune system and this antibody production is supported by a shift towards Th2-type humoral immunity. Second, pregnancy is characterized by the presence of potentially neuroprotective hormones including estrogens, progesterone, and prolactin. The secretion of these factors are thought to play a crucial role for the CNS neuronal and oligodendroglial cell lineages during development (Craig et al., 2003).

From an evolutionary standpoint, biological changes during pregnancy are generally aimed at protecting the fetus and promoting its development. However, the same mechanisms, i.e. suppression of cellular immunity and promotion of neuroprotection, may coincidentally also be highly beneficial for a mother with an autoimmune inflammatory CNS disease. One could therefore consider the advantageous effects in MS a side-effect of pregnancy. Importantly, this ‘side-effect’ can provide valuable insight into MS pathology as well as highlight new therapeutic avenues.

Numerous factors that have been identified in blood during pregnancy have been shown to be immunomodulatory including estrogens, cortisol, progesterone, vitamin D, early pregnancy factor (EPF), α-Fetoprotein and others, some of which also have neuroprotective properties. Estriol is one of the major candidates as a therapeutic agent in MS since it has both potent effects on the immune system as well as the CNS and peaks during the last trimester, i.e. at a time when the most pronounced decrease in relapse rate occurs.
2. Potential mechanisms of sex hormones

2.1. Immunomodulatory properties of sex hormones

2.1.1. Testosterone—The protective role of testosterone in autoimmunity in vivo has been demonstrated by the deleterious effect of castration of male animals on disease susceptibility and severity in numerous models of autoimmune diseases including experimental autoimmune encephalomyelitis (EAE), diabetes in nonobese mice, thyroiditis, and adjuvant arthritis (Ahmed and Penhale, 1982; Bebo et al., 1998; Fitzpatrick et al., 1991; Fox, 1992; Harbuz et al., 1995; Smith et al., 1999). Conversely, testosterone treatment of females can ameliorate a variety of autoimmune disease models (Dalal et al., 1997; Fox, 1992; Sato et al., 1992).

In vitro, naïve T cells stimulated with CNS auto-antigens in the presence of testosterone produce higher levels of IL-5 and IL-10 but decreased levels of IFNγ (Bebo et al., 1999) indicating a Th2-like shift. Similar changes were seen after in vivo treatment of EAE mice with testosterone (Dalal et al., 1997). Studies have also shown that testosterone can reduce the in vitro production of inflammatory cytokines such as TNFα and IL-1β by human macrophages (D’Agostino et al., 1999) and monocytes (Li et al., 1993; Liva and Voskuhl, 2001). These studies further support the hypothesis that testosterone treatment may induce an immune shift in vivo and exert beneficial effects in Th1-mediated autoimmune diseases.

2.1.2. Estrogen—It has been previously shown by numerous laboratories that the clinical severity of both active and adoptive EAE is reduced by estrogen (estriol or 17α-estradiol) treatment in several strains of mice (SJL, C57BL/6, B10.PL, B10.RIII) (Bebo et al., 2001; Ito et al., 2001; Jansson et al., 1994; Kim et al., 1999; Liu et al., 2003; Liu et al., 2002; Matejuk et al., 2001; Polanczyk et al., 2003; Subramanian et al., 2003). Estriol treatment has also been shown to be effective in EAE when administered after disease onset (Kim et al., 1999).

Protective mechanisms of estrogen treatment (both estriol and estradiol) in EAE clearly involve anti-inflammatory processes. Estrogen treatment has been shown to affect cytokines, chemokines, matrix metalloproteinase-9 (MMP-9), antigen presentation and dendritic cell function (Bebo et al., 2001; Ito et al., 2001; Liu et al., 2003; Matejuk et al., 2001; Palaszynski et al., 2004; Subramanian et al., 2003). Estrogen treatment has also recently been shown to induce CD4+CD25+ regulatory T cells in EAE (Matejuk et al., 2004; Polanczyk et al., 2004).

Estrogens regulate gene transcription by nuclear estrogen receptors (ER) and the two nuclear ERs, ERα and ERβ, exhibit distinct transcriptional properties. In addition to the nuclear ERs, plasma membrane-associated ERs mediate the non-genomic signaling pathway. Although both ERα and ERβ are expressed in the immune system and the CNS, studies using ERα signaling deficient mouse strains have shown that clinical protection from EAE by estradiol (Polanczyk et al., 2003) and estriol (Liu et al., 2003) depends on signaling through ERα. Correspondingly, anti-inflammatory mechanisms of estrogens have been found to be mediated by ERα: ERα–selective ligand treatment was sufficient to ameliorate EAE and induced favorable changes in autoantigen-specific cytokine production in the peripheral immune system (decreased TNFα, IFNγ, and IL-6, with increased IL-5) and decreased CNS white matter inflammation and demyelination in EAE (Morales et al., 2006). Selective ERα ligand treatment also decreased CNS infiltration in EAE whereas a selective ERβ ligand had no effect on peripheral cytokine production or CNS infiltration (Tiwari-Woodruff et al., 2007). Estriol treatment effects on MMP-9 bioactivity and CNS infiltration by T cells and monocytes in EAE were also mediated via ERα (Gold et al., 2008b). In addition to these peripheral effects, it has been shown that ERα-mediated regulation of resident CNS cells including microglia is important for amelioration of EAE using a bone-marrow chimera model (Garidou et al., 2004). Overall, these results suggest that the anti-inflammatory effect of estrogens is mediated by ERα.
2.2. Neuroprotective properties of sex hormones

2.2.1. Testosterone—Recently, studies on a possible neuroprotective effect of testosterone have begun to accumulate. Testosterone in its free form can cross the blood-brain-barrier (Iqbal et al., 1983) and thus directly influence neuronal cells. Testosterone has been shown to protect spinal cord neurons in culture from glutamate toxicity (Ogata et al., 1993). Testosterone as well as dehydroteosterone (DHT), which cannot be converted to estrogen, can induce neuronal differentiation and increases in neurite outgrowth in cultured neuronal cells (Lustig, 1994). In addition, testosterone has been shown to protect from oxidative stress in neuronal cell lines (Chisu et al., 2006a; Chisu et al., 2006b). Also, both testosterone and DHT, protected cultured neurons against beta-amyloid toxicity induced cell death and this protective effect of testosterone was not blocked by droloxifene, an estrogen receptor antagonist (Pike, 2001). This indicates that at least some neuroprotective effects of testosterone are not dependent upon conversion to estrogen. While numerous mechanisms of testosterone-mediated neuroprotection may exist, it is possible that some are mediated through an increase in the expression of neurotrophic factors such as brain derived neurotrophic factor (BDNF). Increased survival of neurons during testosterone treatment in the adult avian brain was shown to be abrogated when BDNF was blocked (Rasika et al., 1999). A recent article reviews the neuroprotective effects of testosterone in vitro as well as in vivo in animal models (Bialek et al., 2004).

2.2.2. Estrogen—Numerous reviews have described estrogen’s neuroprotective effects, both in vitro and in vivo (Garcia-Segura et al., 2001; Sribnick et al., 2003; Wise et al., 2001). In vitro, estrogens have been shown to protect neurons in a variety of models of neurodegeneration, including those induced by excitotoxicity and oxidative stress (Behl et al., 1997; Behl et al., 1995; Goodman et al., 1996; Harms et al., 2001). Treatment with estrogen decreased glutamate induced apoptosis and preserved electrophysiologic function in neurons (Sribnick et al., 2004; Zhao et al., 2004). Estrogen treatment also protected oligodendrocytes from cytotoxicity (Cantarella et al., 2004; Sur et al., 2003; Takao et al., 2004) as well as accelerated oligodendrocyte process formation (Zhang et al., 2004). In vivo studies have shown that estrogen treatment can be neuroprotective in animal models of Parkinson’s disease, cerebellar ataxia, late onset leukodystrophy, stroke and spinal cord injury, often by reducing apoptosis (Dubal et al., 2001; Jover et al., 2002; Leranth et al., 2000; Matsuda et al., 2001; Rau et al., 2003; Sierra et al., 2003; Yune et al., 2004). Estrogens have also been shown in vitro to increase dendritic spine formation and synapses on CA1 pyramidal cells of the hippocampus in healthy rats, resulting in improved working spatial memory (Murphy et al., 1998; Rudick and Woolley, 2001; Sandstrom and Williams, 2001; Yankova et al., 2001).

As described above, the anti-inflammatory effects of estrogens in EAE are mediated via ERα. Since anti-inflammatory and neuroprotective effects are not mutually exclusive, it remains possible that some neuroprotective effects may also be mediated through ERβ. However, it is difficult to prove direct neuroprotective effects of ERα ligand treatment in EAE in a setting of such profound anti-inflammatory effects. In contrast, recent data suggest that the ERβ pathway mediates neuroprotective effects in EAE in the absence of an anti-inflammatory effect (Tiwari-Woodruff et al., 2007). In our study, ERα ligand treatment abrogated EAE at the onset and throughout the disease course. In contrast, ERβ ligand treatment had no effect at disease onset but promoted recovery during the chronic phase of the disease and was not anti-inflammatory in the systemic immune system. Also, ERα ligand treatment reduced CNS inflammation, whereas ERβ ligand treatment did not. Interestingly, treatment with either the ERα or the ERβ ligand was neuroprotective, as evidenced by reduced demyelination and preservation of axon numbers in white matter, as well as decreased neuronal abnormalities in gray matter. This is in line with other recent studies using transgenic mice.

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(Rissman et al., 2002) and selective ER\(\beta\) agonists (Rhodes and Frye, 2006) that indicate that the beneficial effects of estrogen on memory function is dependent on the ER\(\beta\) pathway. Selective ER\(\beta\) agonist effects on memory have been linked to increased dendritic branching and upregulation of key synaptic proteins including PSD-95, synaptophysin, and AMPA-receptor subunit GluR1 in the hippocampus (Liu et al., 2008).

3. Sex hormone treatments in MS

3.1. Testosterone

In a pilot clinical trial, ten male MS patients were treated with 10 g of gel containing 100 mg of testosterone in a cross-over design (6 month observation period followed by 12 months of treatment) (Sicotte et al., 2007). Clinical measures of disability and cognition (the Multiple Sclerosis Functional Composite and the 7/24 Spatial Recall Test) were obtained every 3 months. In addition, monthly magnetic resonance imaging measures of enhancing lesion activity and whole brain volumes were acquired. In addition, blood was drawn every three months during the entire study period for immunological studies.

Treatment with testosterone gel was well tolerated and associated with improvement in cognitive performance as measured by the Paced Auditory Serial Addition Task, a test of processing speed and attention widely used in MS. In addition, treatment was associated with a slowing of brain atrophy as measured by MRI. There was no significant effect of testosterone treatment on gadolinium-enhancing lesions (Sicotte et al., 2007). Testosterone treatment also significantly reduced delayed type hypersensitivity (DTH) skin recall responses, a functional \textit{in vivo} measure of inflammatory immune responses, and induced a shift in peripheral lymphocyte composition by decreasing CD4+ T cells and increasing NK cells (Gold et al., 2008a). In addition, PBMC production of IL-2 was significantly decreased while TGF\(\beta\)1 production was increased. Furthermore, PBMCs obtained during the treatment period produced significantly more BDNF and PDGF-BB. The concentrations of BDNF and PDGF-BB in PBMC cultures were in the biologically active range as shown by their ability to reduce glutamate-induced neuronal cell death \textit{in vitro}. These results are consistent with an immunomodulatory as well as a potentially neuroprotective effect of testosterone treatment in MS.

3.2. Estriol

Estriol was administered in a pilot clinical trial to women with MS in an attempt to recapitulate the protective effect of pregnancy on disease (Sicotte et al., 2002). A cross-over study was used whereby patients were followed for 6 months pre-treatment to establish baseline disease activity, which included cerebral MRI every month and neurological examination every 3 months. The patients were then treated with oral estriol (8 mg/day) for 6 months, then observed for 6 more months in the post-treatment period followed by another 4-month re-treatment period. Six RRMS patients and four SPMS patients finished the entire 22 months study period.

As compared with pretreatment baseline, relapsing remitting patients treated with oral estriol (8 mg/day) demonstrated significant decreases in DTH responses. Treatment also decreased gadolinium enhancing lesion numbers and volumes on MRI. When estriol treatment was stopped, enhancing lesions increased to pretreatment levels. When estriol treatment was reinstated, enhancing lesions again were significantly decreased. This improvement in the group as a whole was driven by the beneficial effect of estriol treatment in the RRMS, not the SPMS, group. Interestingly, estriol treatment also significantly increased cognitive function as measured by the PASAT in the RRMS group but not in the SPMS group.

Immunological studies (Soldan et al., 2003) revealed that oral estriol treatment was associated with significant decreases in CD4+ and CD8+ T cells and an increase in CD19+ B cells, with...
no changes in CD64+ monocytes/macrophages. Significant decreases in CD4+CD45Ro+ (memory T cells) and increases in CD4+CD45Ra+ (naive T cells) were also observed. Significantly increased levels of IL-5 and IL-10 and decreased TNFα were observed in stimulated PBMC isolated during estriol treatment. These changes in cytokines correlated with reductions of enhancing lesions on magnetic resonance imaging in RRMS. Further studies were conducted in a subgroup of three of the RRMS patients in this study. Here, supernatants from stimulated PBMCs obtained during treatment showed decreased levels and bioactivity of MMP-9 (Gold et al., 2008b).

4. Conclusion and future directions

A large body of evidence supports the therapeutic potential of testosterone and estrogens in animal models of multiple sclerosis. Mechanisms of action include both immunomodulatory and neuroprotective pathways thus suggesting that sex hormones represent novel treatment options that could beneficially affect the inflammatory as well as the neurodegenerative component of the disease. We now also have first clinical evidence for the effectiveness of testosterone and estriol in MS from two completed pilot studies. As a result, a phase II trial is underway for oral estriol treatment in female patients with RRMS. Both testosterone and estriol have a favorable safety profile in men and women, respectively. Both hormones also have an advantageous route of administration compared to available treatments in MS since testosterone can be applied transdermally and estriol may be taken orally. Thus, these treatments, tailored to each gender, represent an attractive alternative to currently approved therapeutic agents such as interferon-β and glatiramer acetate, which are each taken by injection only.

More research is needed to understand the pathways and mechanisms underlying the beneficial effects of sex hormones on MS pathology. For estrogens, there is accumulating evidence that anti-inflammatory and neuroprotective effects are selectively mediated via ERα and ERβ pathways. One must consider the risk/benefit ratio of any estrogen treatment when considering its use in MS. The goal is to optimize efficacy and minimize toxicity. Hence, determining which estrogen receptor mediates the neuroprotective effect of estrogen treatment is of central importance. The reviewed data demonstrating that treatment with an ERβ ligand is neuroprotective are of clinical relevance, because breast and uterine endometrial cancer are both mediated through ERα, not ERβ. Thus, treatment could be tailored to minimize the risk/benefit ratio for individual patients. If certain conditions such as a known risk for breast or uterine cancer prohibit the use of estriol, the patient may benefit from a standard anti-inflammatory treatment in combination with ERβ ligand treatment. This way, the neuroprotective properties of estrogen treatment could be maintained while avoiding the increased risk of cancer in the breast and uterus.

Comparatively little is known about the anti-inflammatory and neuroprotective mechanisms of testosterone. Testosterone is converted to estrogen in the brain by aromatase, and the neuroprotective properties of testosterone treatment in vivo may be due at least in part to this conversion. However, some studies using the non-convertible dehydrotestosterone (DHT) have also shown testosterone can be directly beneficial.

Testosterone therapy has potentially harmful side effects as it may worsen pre-existing prostate cancer in some men. Testing of prostate specific antigen levels is recommended before and during testosterone therapy. However, testosterone replacement is widely used in aging and hypogonadal men and there is no clear evidence that higher levels of circulating testosterone, within the physiological range, are linked to an increased risk of prostate cancer.
In this review, we have focused on hormonal influences on MS. The gender gap in MS however may be due to effects of sex hormones, genetic differences or a combination of the two. A nonmutually exclusive alternative hypothesis includes a direct genetic effect on the immune system and/or the CNS. That is, specific gene products, which are not induced by gonadal hormones, yet are expressed in a sexually dimorphic manner could induce gender differences in MS pathogenesis and progression. In human studies, these factors cannot be dissected since men and women differ with regard to both sex chromosomes as well as sex hormones. However, there are now sophisticated transgenic mouse models available that allow the examination of effects of sex hormones versus sex chromosomes independently. Recently, our laboratory has employed this model to examine the contribution of gonadal gene complement on immune responses (Palaszynski et al., 2005) as well as susceptibility to autoimmune disease (Smith-Bouvier et al., 2008). Findings suggest that the XX sex chromosome complement, as compared to XY complement, can indeed promote autoimmunity. Taken together, one must consider the contribution of both sex hormones and sex chromosomes in complex autoimmune diseases such as MS.

**Abbreviations**

- BDNF, brain derived neurotrophic factor
- CNS, central nervous system
- DHT, dehydrotestosterone
- DTH, delayed type hypersensitivity
- EAE, experimental autoimmune encephalomyelitis
- ER, estrogen receptor
- IL, interleukin
- IFN, interferon
- MMP, matrix metalloproteinase
- MS, multiple sclerosis
- MRI, magnetic resonance imaging
- NAWM, normal appearing white matter
- PASAT, paced auditory serial addition task
- PBMC, peripheral blood mononuclear cell
- PDGF, Platelet-derived growth factor
- RA, rheumatoid arthritis
- RRMS, relapsing-remitting MS
- SPMS, secondary-progressive MS
- TGF, transforming growth factor
- TNF, tumor necrosis factor

**References**


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