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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

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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 17!-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 dehydrotestosterone (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* and *in vivo* 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 ERα. 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

(Rissman et al., 2002) and selective ERβ agonists (Rhodes and Frye, 2006) that indicate that the beneficial effects of estrogen on memory function is dependent on the ERβ pathway. Selective ERβ 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 *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 TGFβ1 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 *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 reinstituted, 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 nonmutally 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

Abramsky O. Pregnancy and multiple sclerosis. Annals of Neurology 1994;36(Suppl):S38–S41. [PubMed: 7517125]

Ahmed SA, Penhale WJ. The influence of testosterone on the development of autoimmune thyroiditis in thymectomized and irradiated rats. Clin Exp Immunol 1982;48:367–374. [PubMed: 7049452]

Bakshi R, Benedict RH, Bermel RA, Jacobs L. Regional brain atrophy is associated with physical disability in multiple sclerosis: semiquantitative magnetic resonance imaging and relationship to clinical findings. J Neuroimaging 2001;11:129–136. [PubMed: 11296581]

Bebo BF Jr, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA, Offner H. Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. J Immunol 2001;166:2080–2089. [PubMed: 11160259]

Bebo BF Jr, Schuster JC, Vandenbark AA, Offner H. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. Journal of Immunology 1999;162:35–40.

Bebo BF Jr, Zelinka-Vincent E, Adamus G, Amundson D, Vandenbark AA, Offner H. Gonadal hormones influence the immune response to PLP 139–151 and the clinical course of relapsing experimental autoimmune encephalomyelitis. J Neuroimmunol 1998;84:122–130. [PubMed: 9628453]

Behl C, Skutella T, Lezoualc'h F, Post A, Widmann M, Newton CJ, Holsboer F. Neuroprotection against oxidative stress by estrogens: structure-activity relationship. Mol Pharmacol 1997;51:535–541. [PubMed: 9106616]

Behl C, Widmann M, Trapp T, Holsboer F. 17-beta estradiol protects neurons from oxidative stress-induced cell death in vitro. Biochem Biophys Res Commun 1995;216:473–482. [PubMed: 7488136]

Bialek M, Zaremba P, Borowicz KK, Czuczwar SJ. Neuroprotective role of testosterone in the nervous system. Pol J Pharmacol 2004;56:509–518. [PubMed: 15591638]

Birk K, Ford C, Smeltzer S, Ryan D, Miller R, Rudick RA. The clinical course of multiple sclerosis during pregnancy and the puerperium. Arch Neurol 1990;47:738–742. [PubMed: 1972617]

Bo L, Vedeler CA, Nyland H, Trapp BD, Mork SJ. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. Mult Scler 2003;9:323–331. [PubMed: 12926836]

Brex PA, Jenkins R, Fox NC, Crum WR, O'Riordan JI, Plant GT, Miller DH. Detection of ventricular enlargement in patients at the earliest clinical stage of MS. Neurology 2000;54:1689–1691. [PubMed: 10762518]

Cantarella G, Risuglia N, Lombardo G, Lempereur L, Nicoletti F, Memo M, Bernardini R. Protective effects of estradiol on TRAIL-induced apoptosis in a human oligodendrocytic cell line: evidence for multiple sites of interactions. Cell Death Differ. 2004

Catalaa I, Fulton JC, Zhang X, Udupa JK, Kolson D, Grossman M, Wei L, McGowan JC, Polansky M, Grossman RI. MR imaging quantitation of gray matter involvement in multiple sclerosis and its correlation with disability measures and neurocognitive testing. Ajnr Am J Neuroradiol 1999;20:1613–1618. [PubMed: 10543630]

Catalaa I, Grossman RI, Kolson DL, Udupa JK, Nyul LG, Wei L, Zhang X, Polansky M, Mannon LJ, McGowan JC. Multiple sclerosis: magnetization transfer histogram analysis of segmented normal-appearing white matter. Radiology 2000;216:351–355. [PubMed: 10924552]

Chisu V, Manca P, Lepore G, Gadau S, Zedda M, Farina V. Testosterone induces neuroprotection from oxidative stress. Effects on catalase activity and 3-nitro-L-tyrosine incorporation into alpha-tubulin in a mouse neuroblastoma cell line. Arch Ital Biol 2006a;144:63–73. [PubMed: 16642786]

Chisu V, Manca P, Zedda M, Lepore G, Gadau S, Farina V. Effects of testosterone on differentiation and oxidative stress resistance in C1300 neuroblastoma cells. Neuro Endocrinol Lett 2006b;27:807–812. [PubMed: 17187012]

Coles AJ, Cox A, Le Page E, Jones J, Trip SA, Deans J, Seaman S, Miller DH, Hale G, Waldmann H, Compston DA. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. J Neurol 2006;253:98–108. [PubMed: 16044212]

Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group [see comments]. New England Journal of Medicine 1998;339:285–291. [PubMed: 9682040]

Craig A, Ling Luo N, Beardsley DJ, Wingate-Pearse N, Walker DW, Hohimer AR, Back SA. Quantitative analysis of perinatal rodent oligodendrocyte lineage progression and its correlation with human. Exp Neurol 2003;181:231–240. [PubMed: 12781996]

D'Agostino P, Milano S, Barbera C, Di Bella G, La Rosa M, Ferlazzo V, Farruggio R, Miceli DM, Miele M, Castagnetta L, Cillari E. Sex hormones modulate inflammatory mediators produced by macrophages. Ann N Y Acad Sci 1999;876:426–429. [PubMed: 10415638]

Da Silva JA, Spector TD. The role of pregnancy in the course and aetiology of rheumatoid arthritis. Clinical Rheumatology 1992;11:189–194. [PubMed: 1617891]

Dalal M, Kim S, Voskuhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. J Immunol 1997;159:3–6. [PubMed: 9200430]

Damek DM, Shuster EA. Pregnancy and multiple sclerosis. Mayo Clinic Proceedings 1997;72:977–989. [PubMed: 9379704]

De Stefano N, Narayanan S, Matthews PM, Francis GS, Antel JP, Arnold DL. In vivo evidence for axonal dysfunction remote from focal cerebral demyelination of the type seen in multiple sclerosis. Brain 1999;122:1933–1939. [PubMed: 10506094]

Dubal DB, Zhu H, Yu J, Rau SW, Shughrue PJ, Merchenthaler I, Kindy MS, Wise PM. Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. Proc Natl Acad Sci U S A 2001;98:1952–1957. [PubMed: 11172057]

Duquette P, Pleines J, Girard M, Charest L, Senecal-Quevillon M, Masse C. The increased susceptibility of women to multiple sclerosis. Can J Neurol Sci 1992;19:466–471. [PubMed: 1423044]

Evangelou N, Esiri MM, Smith S, Palace J, Matthews PM. Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. Ann Neurol 2000;47:391–395. [PubMed: 10716264]

Filippi M, Bozzali M, Rovaris M, Gonen O, Kesavadas C, Ghezzi A, Martinelli V, Grossman RI, Scotti G, Comi G, Falini A. Evidence for widespread axonal damage at the earliest clinical stage of multiple sclerosis. Brain 2003;126:433–437. [PubMed: 12538409]

Filippi M, Iannucci G, Cercignani M, Assunta RM, Pratesi A, Comi G. A quantitative study of water diffusion in multiple sclerosis lesions and normal-appearing white matter using echo-planar imaging. Arch Neurol 2000a;57:1017–1021. [PubMed: 10891984]

Filippi M, Inglese M, Rovaris M, Sormani MP, Horsfield P, Iannucci PG, Colombo B, Comi G. Magnetization transfer imaging to monitor the evolution of MS: a 1-year follow-up study [In Process Citation]. Neurology 2000b;55:940–946. [PubMed: 11061248]

Fitzpatrick F, Lepault F, Homo-Delarche F, Bach JF, Dardenne M. Influence of castration, alone or combined with thymectomy, on the development of diabetes in the nonobese diabetic mouse. Endocrinology 1991;129:1382–1390. [PubMed: 1874178]

Foster SC, Daniels C, Bourdette DN, Bebo BF Jr. Dysregulation of the hypothalamic-pituitary-gonadal axis in experimental autoimmune encephalomyelitis and multiple sclerosis. J Neuroimmunol 2003;140:78–87. [PubMed: 12864974]

Fox HS. Androgen treatment prevents diabetes in nonobese diabetic mice. J Exp Med 1992;175:1409–1412. [PubMed: 1569406]

Garcia-Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. Prog Neurobiol 2001;63:29–60. [PubMed: 11040417]

Garidou L, Laffont S, Douin-Echinard V, Coureau C, Krust A, Chambon P, Guery JC. Estrogen receptor alpha signaling in inflammatory leukocytes is dispensable for 17beta-estradiol-mediated inhibition of experimental autoimmune encephalomyelitis. J Immunol 2004;173:2435–2442. [PubMed: 15294957]

Gasperini C, Horsfield MA, Thorpe JW, Kidd D, Barker GJ, Tofts PS, MacManus DG, Thompson AJ, Miller DH, McDonald WI. Macroscopic and microscopic assessments of disease burden by MRI in multiple sclerosis: relationship to clinical parameters. J Magn Reson Imaging 1996;6:580–584. [PubMed: 8835949]

Ge Y, Grossman RI, Udupa JK, Wei L, Mannon LJ, Polansky M, Kolson DL. Brain atrophy in relapsing-remitting multiple sclerosis and secondary progressive multiple sclerosis: longitudinal quantitative analysis. Radiology 2000;214:665–670. [PubMed: 10715027]

Gold SM, Chalifoux S, Giesser BS, Voskuhl RR. Immune modulation and increased neurotrophic factor production in multiple sclerosis patients treated with testosterone. J Neuroinflammation 2008a;5:32. [PubMed: 18671877]

Gold SM, Manda SV, Morales LB, Sicotte NL, Voskuhl RR. Estriol treatment reduces matrix metalloprotease-9 activity in multiple sclerosis and experimental autoimmune encephalomyelitis. Mult Scler 2008b;14:S29.

Goodman Y, Bruce AJ, Cheng B, Mattson MP. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. J Neurochem 1996;66:1836–1844. [PubMed: 8780008]

Harbuz MS, Perveen-Gill Z, Lightman SL, Jessop DS. A protective role for testosterone in adjuvant-induced arthritis. Br J Rheumatol 1995;34:1117–1122. [PubMed: 8608351]

Harms C, Lautenschlager M, Bergk A, Katchanov J, Freyer D, Kapinya K, Herwig U, Megow D, Dirnagl U, Weber JR, Hortnagl H. Differential mechanisms of neuroprotection by 17 beta-estradiol in apoptotic versus necrotic neurodegeneration. J Neurosci 2001;21:2600–2609. [PubMed: 11306613]

Hemmer B, Archelos JJ, Hartung HP. New concepts in the immunopathogenesis of multiple sclerosis. Nat Rev Neurosci 2002;3:291–301. [PubMed: 11967559]

Iqbal MJ, Dalton M, Sawers RS. Binding of testosterone and oestradiol to sex hormone binding globulin, human serum albumin and other plasma proteins: evidence for non-specific binding of oestradiol to sex hormone binding globulin. Clin Sci (Lond) 1983;64:307–314. [PubMed: 6681600]

Ito A, Bebo BF Jr, Matejuk A, Zamora A, Silverman M, Fyfe-Johnson A, Offner H. Estrogen treatment down-regulates TNF-alpha production and reduces the severity of experimental autoimmune encephalomyelitis in cytokine knockout mice. J Immunol 2001;167:542–552. [PubMed: 11418693]

Jansson L, Olsson T, Holmdahl R. Estrogen induces a potent suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice. Journal of Neuroimmunology 1994;53:203–207. [PubMed: 8071434]

Jover T, Tanaka H, Calderone A, Oguro K, Bennett MV, Etgen AM, Zukin RS. Estrogen protects against global ischemia-induced neuronal death and prevents activation of apoptotic signaling cascades in the hippocampal CA1. J Neurosci 2002;22:2115–2124. [PubMed: 11896151]

Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology 1999;52:1230–1238. [PubMed: 10214749]

Lassmann H, Bruck W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med 2001;7:115–121. [PubMed: 11286782]

Leranth C, Roth RH, Elswoth JD, Naftolin F, Horvath TL, Redmond DE Jr. Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implications for Parkinson's disease and memory. J Neurosci 2000;20:8604–8609. [PubMed: 11102464]

Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells in vitro. Clin Exp Rheumatol 1993;11:157–162. [PubMed: 8508557]

Liu F, Day M, Muniz LC, Bitran D, Arias R, Revilla-Sanchez R, Grauer S, Zhang G, Kelley C, Pulito V, Sung A, Mervis RF, Navarra R, Hirst WD, Reinhart PH, Marquis KL, Moss SJ, Pangalos MN, Brandon NJ. Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. Nat Neurosci 2008;11:334–343. [PubMed: 18297067]

Liu HB, Loo KK, Palaszynski K, Ashouri J, Lubahn DB, Voskuhl RR. Estrogen receptor alpha mediates estrogen's immune protection in autoimmune disease. J Immunol 2003;171:6936–6940. [PubMed: 14662901]

Liu HY, Buenafe AC, Matejuk A, Ito A, Zamora A, Dwyer J, Vandenbark AA, Offner H. Estrogen inhibition of EAE involves effects on dendritic cell function. J Neurosci Res 2002;70:238–248. [PubMed: 12271473]

Liva SM, Voskuhl RR. Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. J Immunol 2001;167:2060–2067. [PubMed: 11489988]

Losseff NA, Wang L, Lai HM, Yoo DS, Gawne CM, McDonald WI, Miller DH, Thompson AJ. Progressive cerebral atrophy in multiple sclerosis. A serial MRI study. Brain 1996:2009–2019. [PubMed: 9010005]

Lustig RH. Sex hormone modulation of neural development in vitro. Horm Behav 1994;28:383–395. [PubMed: 7729807]

Matejuk A, Adlard K, Zamora A, Silverman M, Vandenbark AA, Offner H. 17beta-estradiol inhibits cytokine, chemokine, and chemokine receptor mRNA expression in the central nervous system of female mice with experimental autoimmune encephalomyelitis. J Neurosci Res 2001;65:529–542. [PubMed: 11550221]

Matejuk A, Bakke AC, Hopke C, Dwyer J, Vandenbark AA, Offner H. Estrogen treatment induces a novel population of regulatory cells, which suppresses experimental autoimmune encephalomyelitis. J Neurosci Res 2004;77:119–126. [PubMed: 15197745]

Matsuda J, Vanier MT, Saito Y, Suzuki K. Dramatic phenotypic improvement during pregnancy in a genetic leukodystrophy: estrogen appears to be a critical factor. Hum Mol Genet 2001;10:2709–2715. [PubMed: 11726558]

McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol 2007;8:913–919. [PubMed: 17712344]

Morales LB, Loo KK, Liu HB, Peterson C, Tiwari-Woodruff S, Voskuhl RR. Treatment with an estrogen receptor alpha ligand is neuroprotective in experimental autoimmune encephalomyelitis. J Neurosci 2006;26:6823–6833. [PubMed: 16793889]

Murphy DD, Cole NB, Greenberger V, Segal M. Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. J Neurosci 1998;18:2550–2559. [PubMed: 9502814]

Narayanan S, Fu L, Pioro E, De Stefano N, Collins DL, Francis GS, Antel JP, Matthews PM, Arnold DL. Imaging of axonal damage in multiple sclerosis: spatial distribution of magnetic resonance imaging lesions. Ann Neurol 1997;41:385–391. [PubMed: 9066360]

Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA. Remission of rheumatoid arthritis during pregnancy and maternal-fetal class II alloantigen disparity. American Journal of Reproductive Immunology 1992;28:226–227. [PubMed: 1285885]

Ogata T, Nakamura Y, Tsuji K, Shibata T, Kataoka K. Steroid hormones protect spinal cord neurons from glutamate toxicity. Neuroscience 1993;55:445–449. [PubMed: 8104320]

Orton SM, Herrera BM, Yee IM, Valdar W, Ramagopalan SV, Sadovnick AD, Ebers GC. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol 2006;5:932–936. [PubMed: 17052660]

Palaszynski KM, Liu H, Loo KK, Voskuhl RR. Estriol treatment ameliorates disease in males with experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neuroimmunol 2004;149:84–89. [PubMed: 15020068]

Palaszynski KM, Smith DL, Kamrava S, Burgoyne PS, Arnold AP, Voskuhl RR. A Yin-Yang Effect Between Sex Chromosome Complement and Sex Hormones on the Immune Response. Endocrinology. 2005

Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 2001;50:389–400. [PubMed: 11558796]

Pike CJ. Testosterone attenuates beta-amyloid toxicity in cultured hippocampal neurons. Brain Res 2001;919:160–165. [PubMed: 11689174]

Polanczyk M, Zamora A, Subramanian S, Matejuk A, Hess DL, Blankenhorn EP, Teuscher C, Vandenbark AA, Offner H. The protective effect of 17beta-estradiol on experimental autoimmune encephalomyelitis is mediated through estrogen receptor-alpha. Am J Pathol 2003;163:1599–1605. [PubMed: 14507666]

Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H. Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol 2004;173:2227–2230. [PubMed: 15294932]

Rasika S, Alvarez-Buylla A, Nottebohm F. BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. Neuron 1999;22:53–62. [PubMed: 10027289]

Rau SW, Dubal DB, Bottner M, Gerhold LM, Wise PM. Estradiol attenuates programmed cell death after stroke-like injury. J Neurosci 2003;23:11420–11426. [PubMed: 14673006]

Rhodes ME, Frye CA. ERbeta-selective SERMs produce mnemonic-enhancing effects in the inhibitory avoidance and water maze tasks. Neurobiol Learn Mem 2006;85:183–191. [PubMed: 16326119]

Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA. Disruption of estrogen receptor beta gene impairs spatial learning in female mice. Proc Natl Acad Sci U S A 2002;99:3996–4001. [PubMed: 11891272]

Rudick CN, Woolley CS. Estrogen regulates functional inhibition of hippocampal CA1 pyramidal cells in the adult female rat. J Neurosci 2001;21:6532–6543. [PubMed: 11517242]

Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. Neurology 1999;53:1698–1704. [PubMed: 10563615]

Runmarker B, Andersen O. Pregnancy is associated with a lower risk of onset and a better prognosis in multiple sclerosis [see comments]. Brain 1995;118:253–261. [PubMed: 7895009]

Sandstrom NJ, Williams CL. Memory retention is modulated by acute estradiol and progesterone replacement. Behav Neurosci 2001;115:384–393. [PubMed: 11345963]

Santos AC, Narayanan S, de Stefano N, Tartaglia MC, Francis SJ, Arnaoutelis R, Caramanos Z, Antel JP, Pike GB, Arnold DL. Magnetization transfer can predict clinical evolution in patients with multiple sclerosis. J Neurol 2002;249:662–668. [PubMed: 12111296]

Sato EH, Ariga H, Sullivan DA. Impact of androgen therapy in Sjogren's syndrome: hormonal influence on lymphocyte populations and Ia expression in lacrimal glands of MRL/Mp-lpr/lpr mice. Invest Ophthalmol Vis Sci 1992;33:2537–2545. [PubMed: 1634351]

Sicotte NL, Giesser BS, Tandon V, Klutch R, Steiner B, Drain AE, Shattuck DW, Hull L, Wang HJ, Elashoff RM, Swerdloff RS, Voskuhl RR. Testosterone treatment in multiple sclerosis: a pilot study. Arch Neurol 2007;64:683–688. [PubMed: 17502467]

Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TC, Voskuhl RR. Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol 2002;52:421–428. [PubMed: 12325070]

Sierra A, Azcoitia I, Garcia-Segura L. Endogenous estrogen formation is neuroprotective in model of cerebellar ataxia. Endocrine 2003;21:43–51. [PubMed: 12777702]

Smith ME, Eller NL, McFarland HF, Racke MK, Raine CS. Age dependence of clinical and pathological manifestations of autoimmune demyelination. Implications for multiple sclerosis. Am J Pathol 1999;155:1147–1161. [PubMed: 10514398]

Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, Arnold AP, Singh RR, Voskuhl RR. A role for sex chromosome complement in the female bias in autoimmune disease. J Exp Med 2008;205:1099–1108. [PubMed: 18443225]

Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol 2003;171:6267–6274. [PubMed: 14634144]

Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005;23:683–747. [PubMed: 15771584]

Sribnick EA, Ray SK, Nowak MW, Li L, Banik NL. 17beta-estradiol attenuates glutamate-induced apoptosis and preserves electrophysiologic function in primary cortical neurons. J Neurosci Res 2004;76:688–696. [PubMed: 15139027]

Sribnick EA, Wingrave JM, Matzelle DD, Ray SK, Banik NL. Estrogen as a neuroprotective agent in the treatment of spinal cord injury. Ann N Y Acad Sci 2003;993:125–133. [PubMed: 12853305] discussion 159-60

Stevenson VL, Leary SM, Losseff NA, Parker GJ, Barker GJ, Husmani Y, Miller DH, Thompson AJ. Spinal cord atrophy and disability in MS: a longitudinal study. Neurology 1998;51:234–238. [PubMed: 9674808]

Subramanian S, Matejuk A, Zamora A, Vandenbark AA, Offner H. Oral feeding with ethinyl estradiol suppresses and treats experimental autoimmune encephalomyelitis in SJL mice and inhibits the recruitment of inflammatory cells into the central nervous system. J Immunol 2003;170:1548–1555. [PubMed: 12538720]

Sur P, Sribnick EA, Wingrave JM, Nowak MW, Ray SK, Banik NL. Estrogen attenuates oxidative stress-induced apoptosis in C6 glial cells. Brain Res 2003;971:178–188. [PubMed: 12706234]

Swerdloff RS, Wang C. Androgens and the ageing male. Best Pract Res Clin Endocrinol Metab 2004;18:349–362. [PubMed: 15261842]

Takao T, Flint N, Lee L, Ying X, Merrill J, Chandross KJ. 17beta-estradiol protects oligodendrocytes from cytotoxicity induced cell death. J Neurochem 2004;89:660–673. [PubMed: 15086523]

Tiwari-Woodruff S, Morales LB, Lee R, Voskuhl RR. Differential neuroprotective and antiinflammatory effects of estrogen receptor (ER)alpha and ERbeta ligand treatment. Proc Natl Acad Sci U S A 2007;104:14813–14818. [PubMed: 17785421]

Tortorella C, Viti B, Bozzali M, Sormani MP, Rizzo G, Gilardi MF, Comi G, Filippi M. A magnetization transfer histogram study of normal-appearing brain tissue in MS. Neurology 2000;54:186–193. [PubMed: 10636146]

Verdru P, Theys P, D'Hooghe MB, Carton H. Pregnancy and multiple sclerosis: the influence on long term disability. Clin Neurol Neurosurg 1994;96:38–41. [PubMed: 8187380]

Voskuhl RR. Sex differences in autoimmune diseases. Horm Brain Behav. in press

Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C. The Pregnancy In Multiple Sclerosis, G. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain 2004;127:1353–1360. [PubMed: 15130950]

Wei T, Lightman SL. The neuroendocrine axis in patients with multiple sclerosis. Brain 1997;120(Pt 6): 1067–1076. [PubMed: 9217689]

Weinshenker BG. Natural history of multiple sclerosis. Ann Neurol 1994;36(Suppl):S6–S11. [PubMed: 8017890]

Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. Science 1999;283:1277–1278. [PubMed: 10084932]

Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M. Minireview: neuroprotective effects of estrogen-new insights into mechanisms of action. Endocrinology 2001;142:969–973. [PubMed: 11181507]

Yankova M, Hart SA, Woolley CS. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: a serial electron-microscopic study. Proc Natl Acad Sci U S A 2001;98:3525–3530. [PubMed: 11248111]

Yune TY, Kim SJ, Lee SM, Lee YK, Oh YJ, Kim YC, Markelonis GJ, Oh TH. Systemic administration of 17beta-estradiol reduces apoptotic cell death and improves functional recovery following traumatic spinal cord injury in rats. J Neurotrauma 2004;21:293–306. [PubMed: 15115604]

Zhang Z, Cerghet M, Mullins C, Williamson M, Bessert D, Skoff R. Comparison of in vivo and in vitro subcellular localization of estrogen receptors alpha and beta in oligodendrocytes. J Neurochem 2004;89:674–684. [PubMed: 15086524]

Zhao L, Wu TW, Brinton RD. Estrogen receptor subtypes alpha and beta contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. Brain Res 2004;1010:22–34. [PubMed: 15126114]