Sex-Based Differences in Multiple Sclerosis (Part I): Biology of Disease Incidence

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Abstract Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease that leads to neuron damage and progressive disability. One major feature of multiple sclerosis (MS) is that it affects women three times more often than men. In this chapter, we overview the evidence that the autoimmune component of MS, which predominates in the early stages of this disease, is more robust in women than in men and undergoes a sharp increase with the onset of puberty. In addition, we discuss the common rodent models of MS that have been used to study the sex-based differences in the development of central nervous system (CNS) autoimmunity. We then address the biological underpinnings of this enhanced MS risk in women by first reviewing the autoimmune mechanisms that are thought to lead to the initiation of this disease and then honing in on how these mechanisms differ between the sexes. Finally, we review what is known about the hormonal and genetic basis of these sex differences in CNS autoimmunity.

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1 Introduction

It is well recognized that a number of autoimmune diseases including MS are more common in women (Whitacre 2001). For MS, three times more women are affected with this disease than men, making female sex one of the top MS risk factors (Orton et al. 2006). There is also evidence that women are more likely to be diagnosed with MS than men after experiencing an incident demyelinating event (Dobson et al. 2012) and that the "feminine" version of this disease involves more frequent bouts or relapses (Tremlett et al. 2008; Kalincik et al. 2013). This chapter will review this evidence and will introduce the animal models that have been used to study sex differences in CNS autoimmunity. Furthermore, it will address how the autoimmune mechanisms that are thought to lead to MS initiation are more robust in women and what features of the immune system exhibit a sex dichotomy. Finally, the underlying hormonal and genetic basis of these sex differences will be discussed.

2 Sex-Based Differences in MS Incidence and Early Disease Activity

When considering the different MS forms, it is apparent that only "bout-onset" or relapsing-remitting MS (RRMS) is more common in women, while "progressive-onset" disease (or primary progressive MS, PPMS) affects women and men equally (Runmarker and Andersen 1993; Broman et al. 1981; Thompson et al. 1997). This section will thus focus on sex differences in RRMS.

2.1 Female Preponderance of RRMS Manifests Post-puberty

The incidence of RRMS is highest in the reproductive years, with a peak age of onset of 29 years in women and 31 years in men (Cossburn et al. 2012). Comparatively, pediatric-onset MS (onset 16 years or less) and late-onset MS (onset >50 years) are less frequent, each accounting for between 2–10 % of cases (Sindern et al. 1992; Ghezzi et al. 1997; Duquette et al. 1987; Pohl et al. 2007; Banwell et al. 2007; Bove et al. 2012; Tremlett and Devonshire 2006; Polliack et al. 2001; Noseworthy et al. 1983; Kis et al. 2008). The female-to-male ratio (F:M) of MS also varies across the life span, affecting females and males equally prior to 10 years (F:M= 0.8–1.4:1), undergoing a sharp increase post-puberty (F:M of 2–3:1), and a modest decline after 50 years (F:M= 1.4–1.9 to 1) (Sindern et al. 1992; Ghezzi et al. 1997; Duquette et al. 1987; Pohl et al. 2007; Banwell et al. 2007; Bove et al. 2012; Tremlett and Devonshire 2006; Polliack et al. 2001; Noseworthy et al. 1983; Kis et al. 2008).

The sharp increase in the incidence of MS and the female preponderance of MS with puberty suggests that alterations in gonadotrophin or gonadal hormone levels are enhancing autoimmune mechanisms, particularly in females. Indeed, a number of recent epidemiological studies have implicated a role specifically for female pubertal factors in MS risk (Ramagopalan et al. 2009; Sloka et al. 2006; Ahn et al. 2014). One case-control study reported finding a significant relationship between having an earlier age of pubertal onset and MS risk in women, but not men (Ramagopalan et al. 2009). In addition, the age of menarche was found to positively associate with the age of onset of first symptoms in female RRMS patients, further supporting the notion that puberty in females serves as a point of inflection of increased MS risk (Sloka et al. 2006). Most recently, a prospective study conducted by the Canadian Pediatric Demyelinating Disease group investigated the relationship between age of menarche and MS outcomes in children who have experienced a first demyelinating event (called acute demyelinating syndrome, ADS) (Ahn et al. 2014). It was observed that female ADS children who had a later age of menarche were ∼40 % less likely to be diagnosed with MS, even after adjusting for a number of factors that are known to be predictive of MS diagnosis in this population (Ahn et al. 2014). Notably, one factor that could not be separated out from age of menarche in

these studies is body mass index since these variables are along the same biological pathway, and menarche only occurs in girls once they achieve a critical weight (Johnston et al. 1971). Thus, it is possible that increased adiposity, which is another MS risk factor in children (Munger et al. 2009, 2013; Langer-Gould et al. 2013), is a driver of these puberty-associated effects on MS outcomes. In summary, pubertal onset appears to be a point of inflection of MS risk, particularly in females, which may relate either to increased adiposity or puberty-associated elevations in pituitary or gonadal hormones. How gonadal hormones and gonadotrophins influence the immune system to increase MS risk will be further discussed in Sect. 6.

2.2 Sex-Based Differences in Early Inflammatory Disease Activity in RRMS

Insights into sex-based differences in early disease activity in RRMS can also be gained by comparing certain disease features between women and men including (1) the risk of MS diagnosis after the first demyelinating event, (2) the number of inflammatory or T1-weighted gadolinium (Gd)-enhancing lesions upon magnetic resonance imaging (MRI), and (3) the frequency of MS relapses. Coinciding with the notion that individuals more robust CNS autoimmune mechanisms operate in females, it is reported that who have experienced an incident demyelinating event are more likely to go on to experience a second event if they are women rather than if they are men (Dobson et al. 2012). This is particularly true for patients who present with optic neuritis, where the risk of subsequent MS diagnosis is twofold–fourfold higher in women than in men (Swanton et al. 2010; Rizzo and Lessell 1988).

One of the best MRI correlates of relapses in MS is the presence of Gd-enhancing lesions in the brain (Kappos et al. 1999), which indicates disruption of the blood-brain barrier (BBB) (Bruck et al. 1997). There are a number of studies that compared the number of Gd-enhancing lesions in male and female MS patients. Most (Weatherby et al. 2000; Pozzilli et al. 2003; Tomassini et al. 2005) but not all (Barkhof et al. 2005) of these studies reported finding a higher number of Gd-enhancing lesions in scans of women versus men. First, Weatherby et al. (2000) in a small cross-sectional study of male and female MS patients (29 RRMS/21 secondary progressive MS, SPMS) reported that female MS patients exhibited a 2.5-fold higher number of Gd-enhancing lesions than men with this disease (Weatherby et al. 2000). Similar findings were reported by Pozilli and colleagues, first in a study of 413 MS patients (266 RRMS/47 SPMS) (Pozzilli et al. 2003) and then in a subsequent study of 60 RRMS patients (Tomassini et al. 2005). However, a more recent, larger-scale study of 1,328 MS patients in the Sylvia Lawry Centre for MS Research (SLCMSR) database that included patients from placebo control arms of randomized clinical trials and natural history studies showed only a tendency ($p = 0.20$) for a higher number of Gd-enhancing lesions in women (Barkhof et al. 2005).

A major limitation of imaging studies is that they are often cross-sectional in nature and therefore only capture a "snapshot" of disease activity in time. Measuring relapse rates in larger patient groups (>350 patients) longitudinally have provided another indicator of the activity of peripherally driven autoimmune mechanisms in early MS. Indeed, analysis of patient data pooled from placebo arms of clinical trials in the SLCMSR database showed that women with relapsing-onset MS exhibited higher relapse rates than men (Held et al. 2005). This finding was subsequently validated in two larger retrospective studies, one of the 2,477 RRMS patients in the British Columbia MS database, which reported a 14.4 % higher relapse rate in women than men (Tremlett et al. 2008), and a second study of 11,570 RRMS patients in the MSbase study group, which reported an 18.8 % higher relapse rate in women (Kalincik et al. 2013). These latter studies reported that this higher relapse rate in women was still apparent after adjusting for the use of disease modifying agents (Tremlett et al. 2008; Kalincik et al. 2013). Interestingly, the study by the MSbase group also found that the F-to-M ratio of MS was higher in the subset of patients who showed the highest relapse rate in the first four years of disease (Kalincik et al. 2013), further indicating that more frequent relapse activity defines a more "feminine" version of this disease. Together with the MRI studies, these data suggest a trend toward higher inflammatory disease activity in female than in male patients in early RRMS.

3 Modeling Sex-Based Differences in CNS Autoimmunity in Mice

Experimental autoimmune encephalomyelitis (EAE) is the most common animal model of MS (Gold et al. 2006). This classic T helper (Th) cell-mediated disease is induced in mammals by vaccination with protein components of the myelin sheath emulsified with complete Freunds' adjuvant (CFA) (i.e., *active EAE*) (Stromnes and Goverman 2006a). EAE is most commonly induced in mice, and most strains of mice with the exception of SJL require injection of pertussis toxin to break tolerance. Pertussis toxin is as an additional adjuvant that serves to boost adaptive T-cell responses and to activate the BBB (Wakatsuki et al. 2003; Kerfoot et al. 2004). EAE can also be induced by transferring activated myelin-reactive Th cells from mice that have EAE into healthy mice (i.e., *adoptive transfer EAE*) (Stromnes and Goverman 2006b) and can also occur *spontaneously* in mice that have been engineered to overexpress a T-cell receptor (TCR) that is specific for myelin antigens (Goverman et al. 1993; Bettelli et al. 2003).

EAE in rodents is considered to be useful in modeling the initiation of Th cell-mediated mechanisms that precipitate the initial attack of CNS autoimmunity (Gold et al. 2006). In rodents, the focal lesions that occur in the spinal cord and cerebellum in EAE resemble the acute lesions that are found in the brain in RRMS patients, in that they display a perivascular location and show T-cell and macrophage infiltration

with focal demyelination and axon loss (Gold et al. 2006). Although there exist distinct differences in the pathology between rodent EAE and MS (i.e., MS involves a greater CNS recruitment of CD8$^+$ T cells and a more brain-focused inflammatory response than in EAE) (Babbe et al. 2000; Kap et al. 2010), these differences can be overcome by inducing EAE in the marmoset, a non-human primate that is more genetically similar to humans and is conventionally housed and thus naturally infected with viruses including Epstein-Barr virus (Kap et al. 2010). Nonetheless, because of the high costs and ethical restrictions associated with research in non-human primates, traditional rodent EAE models remain the gold standard for modeling how factors such as sex and environment can impact disease risk.

A number of rodent strains have been reported to exhibit a female bias in disease development. For instance, EAE induced in the Lewis rat with guinea pig spinal cord homogenate and CFA manifests as a monophasic disease in males and a relapsing–remitting disease in females (Keith 1978). A female-biased disease is also observed in ASW and NZW mouse strains as well as the highly EAE-susceptible SJL mouse strain, which has since become the preferred model for studying sex differences in CNS autoimmunity (Papenfuss et al. 2004). Depending on the mode of vaccination or EAE induction, young adult female SJL mice can develop either a higher incidence of EAE (Papenfuss et al. 2004; Cua et al. 1995), or like Lewis rats have a higher propensity to relapse (Bebo et al. 1996), than male counterparts. Furthermore, it has been also shown that transfer of female vs. male myelin-reactive SJL T-cell lines also induces more severe EAE in recipients, than transfer of male T-cell lines indicating that Th cells are major drivers of this sex difference (Bebo et al. 1999; Voskuhl et al. 1996). Notably, a sex bias in EAE is not observed in all mouse strains. Some commonly used inbred strains such as C57BL6 and B10.Pl show no sex difference in EAE development or show slightly more severe EAE in males (Papenfuss et al. 2004). It has been speculated that these differences are related to the higher susceptibility of male mice to pertussis toxin (Papenfuss et al. 2004) or to genetic differences between strains (Smith-Bouvier et al. 2008; Case et al. 2013). In sum, there are a number of rodent MS models that exhibit a female bias in EAE development and can be useful to study the underlying biology of the more robust CNS autoimmune attacks observed in females.

4 Immune Pathogenesis of MS Onset

Before discussing how sex-based differences in immunity contribute to MS, it is important to first review what is known about the immune pathogenesis of MS initiation. Genome-wide association studies in MS recently identified a number of single nucleotide polymorphisms beyond those in the HLA region that associate with MS (Sawcer et al. 2011). Most of these polymorphisms lie in close proximity to genes involved in common pathways of antigen presentation, T regulatory function, Th-cell activation, and cytokine production (Sawcer et al. 2011), validating the notion that MS is a Th cell-driven autoimmune disease. The current view

is that the incident attack in RRMS and early relapses in the disease are brought about when myelin-activated Th cells get activated in the periphery, expand, and traffic across the BBB into the CNS (Prat and Martin 2002; Petermann and Korn 2011). Once in the CNS, they re-encounter myelin presented by microglia and/or other antigen-presenting cells (APC), which triggers the secretion of pro-inflammatory cytokines and chemokines and the subsequent influx of other immune cell types into the CNS (B cells, CD8^{+} T cells, monocytes, neutrophils, etc.) (Prat and Martin 2002; Petermann and Korn 2011). It is the culminated action of these immune cells and their immune products that lead to myelin and axon damage in the acute MS lesion (Prat and Martin 2002; Petermann and Korn 2011).

Both MS patients and healthy people have myelin-specific Th cells in their circulation (Prat and Martin 2002). So why do these cells get activated in some people and not in others? By the established rules of T-cell engagement, a self-reactive Th cell should not be activated unless it sees both antigen in the context of MHC Class II (signal 1) and a co-stimulatory signal such as CD80 or CD86 on the same APC (signal 2) (Steinman et al. 2003). Signal 2 is only upregulated during infection or upon stimulation of pattern recognition receptors such as Toll-like receptors (Steinman et al. 2003). Thus, a commonly held view is that myelin-reactive Th cells become activated during MS because they recognize a myelin-like, cross-reactive epitope in the context of a microbial infection (Prat and Martin 2002). Proof of this concept is provided by studies that used "humanized" mice that express both a myelin basic protein (MBP)-specific TCR that is restricted by the MS risk allele HLA DR2b (MBP 85-89) and HLA DRB2b itself (Harkiolaki et al. 2009). It has been shown that EAE can be induced in these mice by infecting them with certain pathogens that express proteins with amino acid sequence homology to MBP 85-89 (Harkiolaki et al. 2009).

In addition to microbial involvement, immune and genetic studies have also raised the possibility that key peripheral tolerance mechanisms may be defective in MS. In particular, it has been found that FoxP3^{+}CD4^{+}CD25^{hi} T regulatory cells (Treg) taken from MS patients, though not present at a different frequency in peripheral blood, are less effective than Treg from healthy controls at suppressing the proliferation of Th effector cells in co-culture (Viglietta et al. 2004; Venken et al. 2008; Haas et al. 2005; Feger et al. 2007; Cerosaletti et al. 2013). Part of these defects in MS Treg suppression relate to a decreased ability of these cells to respond to interleukin (IL)-2, a factor that is critical for Treg survival, expansion, and FoxP3 expression (Venken et al. 2008; Cerosaletti et al. 2013). In addition, it is reported that Treg from MS patients show a more restricted Vbeta repertoire than Treg from healthy people and that a lower frequency of these MS Treg are recent thymic emigrants (Haas et al. 2007). Given that newly minted Tregs exhibit a greater suppressive capacity than memory Treg (Haas et al. 2007), these findings further explain why the MS Treg population is less functional.

Of the different Th cell types (Th1, Th2, or Th17), current evidence indicates that Th1 cells (that secrete IFNγ) and Th17 cells (that secrete IL-17A) are involved in MS pathogenesis (Petermann and Korn 2011). On the other hand, anti-inflammatory Th2 cells are proposed to balance these pro-inflammatory responses

(Petermann and Korn 2011). Indeed, both transcripts and protein products of IFNγ and IL-17 have been detected within active MS lesions and in the CSF of MS patients (Balashov et al. 1999; Kebir et al. 2009; Tzartos et al. 2008; Lock et al. 2002). Further evidence in support of an involvement of Th1 cells in MS is that IFNγ-producing T cells are detected at a higher frequency in the blood of MS patients just prior to acute attacks (Beck et al. 1988) and treatment with IFNγ causes relapses in MS (Panitch et al. 1987). On the other hand, a recent report that anti-IL-17A therapy (secukinumab) was effective at reducing inflammatory lesions in a small phase I trial in RRMS (Havrdová et al. 2012) also implicates Th17-effector mechanisms in this disease.

Studies in humans and in mice have illuminated the potential mechanisms of how Th1 and Th17 cells mediate inflammation and tissue damage in MS and EAE. IFNγ produced by Th1 cells promotes the class switching of myelin-specific antibodies to complement-fixing IgG2a and IgG3 types (Young and Hardy 1995), increases ICAM-1 expression on vascular endothelium of the BBB (Kebir et al. 2009), and upregulates MHC Class I and Class II and co-stimulatory markers on microglia, thus enhancing their ability to present myelin antigen in the CNS (Shrikant and Benveniste 1996). IFNγ also triggers myeloid cells to produce pro-inflammatory cytokines (IL-12, TNFα) and reactive oxygen and nitrogen species (Young and Hardy 1995; Shrikant and Benveniste 1996), which can be toxic to oligodendrocytes and neurons. Finally, IFNγ triggers the production of CCL2 by microglia (Tran et al. 2000) and this chemokine is critical for the CNS recruitment of CCR2$^+$ inflammatory monocytes (Huang et al. 2001).

Studies in mice have also provided support for a pathogenic role for Th17 cells in disease. Adoptive transfer of IL-23-polarized myelin-reactive Th17 cell lines, like IL-12-polarized Th1 cell lines, can induce EAE; however, these Th17 cell lines evoke a different CNS inflammatory cascade that is dominated instead by neutrophilic inflammation and associated with more extensive tissue damage (Kroenke et al. 2008), in part through a possible ability of Th17 cells to damage axons directly (Siffrin et al. 2010). Fate-mapping studies of Th17 cells in mice during EAE have indicated that these cells have a competitive advantage in accessing the CNS (Hirota et al. 2011), and once there start to co-produce IFNγ (Hirota et al. 2011; Duhen et al. 2013). Indeed, T cells co-producing IFNγ and IL-17A have been detected in the acute lesions in MS (Kebir et al. 2009) and as a group, these T cells are even more highly pathogenic than "pure" Th1 and Th17 cells due to their higher potential to cross the BBB (Kebir et al. 2009), higher production of pro-inflammatory cytokines GM-CSF, IL-22, and granzyme (Kebir et al. 2009; Duhen et al. 2013), and higher capacity to elicit production of IL-6 and IL-1 by microglia (Murphy et al. 2010).

5 Sex Differences in Immune Functioning and Trafficking That May Explain the Female Preponderance of MS

To understand why females have a higher MS incidence, it is essential to understand how peripheral tolerance mechanisms and Th immune responses are different between males and females and whether there exist sex differences in the ability of T cells to cross the BBB or of CNS-resident APC to reprime myelin-reactive Th cells once they reach the CNS. Additionally, given the recently recognized role for B cells in mediating relapses in MS, it is also important to address the sex differences in B-cell biology.

5.1 Sex Differences in Treg Numbers and Function

In healthy humans, it has been reported that males exhibit a higher number of Treg than females in peripheral blood (Afshan et al. 2012). A similar trend for higher Treg in males has been reported for SJL mice in the spleen (Hussain et al. 2011). However, no one has yet investigated whether Treg numbers differ at the site of autoimmune attack in MS or EAE. A number of groups have compared the Treg suppressive capacities of male and female murine CD4$^+$CD25^{high} cells, and although one study did report a higher IL-10 production by CD4$^+$CD25^{high} cells in male SJL mice (Hussain et al. 2011), this study and other reports did not observe sex differences in Treg function using in vitro suppressor assays (Hussain et al. 2011; Reddy et al. 2005; Cho et al. 2013). Furthermore, although FoxP3 is encoded on the X chromosome, this gene does not escape X inactivation and is expressed at the same gene dosage in male and female cells (Carrel and Willard 2005). Thus, at present, there is no strong evidence to suggest that sex differences in Treg functioning account for the sex differences in CNS autoimmunity.

5.2 Sex Differences in T-Cell Numbers and Proliferative Capacity

Women exhibit higher numbers of Th (CD4$^+$) T cells in peripheral blood as compared to men (Amadori et al. 1995), and these higher numbers likely relate to a higher thymic T-cell output in females (Pido-Lopez et al. 2001). Studies in mice have indicated that this sex difference in thymic output is caused by a suppressive effect of androgens on thymocyte development (Eidinger and Garrett 1972). In addition to basal differences in T cell numbers, female T cells are known to expand more robustly than male cells upon antigenic stimulation (Weinstein et al. 1984). This distinguishing feature of female T cells has been observed both in the context of murine EAE (Kim and Voskuhl 1999; Zhang et al. 2012) and in humans after

administration of a not an herpes simplex virus vaccine (Zhang et al. 2008). Greer et al. (2004) also provided supportive evidence that women exhibit a more robust Th expansion during MS (Greer et al. 2004). They found that peripheral blood mononuclear cells (PBMC) collected from female MS patients and healthy controls exhibited a twofold higher proliferation rate as compared to male counterparts toward the immunodominant epitope of proteolipid protein (PLP) (Wilcoxen et al. 2000).

Although the precise cellular mechanism explaining why female T cells proliferate more than male T cells is not known, studies in mice suggest that it relates to sex-based differences in both the intrinsic activation potential of the T-cell and APC functioning (Weinstein et al. 1984; Zhang et al. 2012; Wilcoxen et al. 2000). Indeed, murine female $CD4^+$ T cells proliferate more robustly than male $CD4^+$ T cells even when cultured in isolation with submaximal amounts of anti-CD3 and anti-CD28 (Zhang et al. 2012). In addition, several groups have reported that female but not male murine APC (either macrophages or dendritic cells) have a stimulatory effect on Th-cell proliferation in co-cultures (Weinstein et al. 1984; Zhang et al. 2012; Wilcoxen et al. 2000). What the APC are doing in this context is not completely clear; however, it is reported that female macrophages produce higher levels of IL-12 and lower levels of IL-10 as compared with male APC (Wilcoxen et al. 2000). IL-12 would support the growth of Th1 cells, which are also found to be more abundant in women (see Sect. 5.3), while IL-10 would inhibit T-cell growth and Th differentiation by downregulating MHC Class II and co-stimulatory expression on APC (Buelens et al. 1995). Altogether, these studies suggest that one reason why females may be more likely to develop MS is because female autoreactive T cells expand more robustly than male counterparts upon encountering antigen and are more likely to trigger the initiation of disease.

5.3 Female Th Cells are Biased Toward Th1, While Male Th Cells are Biased Toward Th2 or Th17

In addition to expanding more robustly, $CD4^+$ T cells from females are more biased toward Th1 cytokine production as compared to male $CD4^+$ T cells. The higher propensity of females to produce the Th1 cytokine IFNγ was first reported in 1984 in a study that examined cytokine responses in mice to Bacille de Calmette et Guerin (i.e., BCG) vaccination (Huygen and Palfliet 1984). This sex difference was later observed in EAE, where it was noted that a higher production of IFNγ by myelin-reactive T cells in the periphery was the main feature that correlated with the more severe active or adoptive transfer EAE in the female sex (Cua et al. 1995; Bebo et al. 1998). A female Th1 bias in cytokine production has also been observed in the context of MS in a series of studies by Pelfrey and colleagues (Pelfrey et al. 2002; Moldovan et al. 2008). Using ELISPOT assay, they investigated the production of

cytokines by PBMC obtained from MS patients and healthy controls after stimulation with myelin antigens, vaccine-relevant antigens (e.g., tetanus toxoid, diptheria toxoid), or polyclonal stimuli such as phytohaemagglutinin or anti-CD3 (Pelfrey et al. 2002; Moldovan et al. 2008). They found that female MS patients exhibited a higher frequency of cells secreting the Th1 cytokine IFNγ and a lower frequency of cells secreting the Th2 cytokine IL-5 in peripheral blood as compared to male MS patients when PBMC were pulsed with certain PLP peptides and vaccination-related antigens (tetanus and diptheria toxoid), (Pelfrey et al. 2002; Moldovan et al. 2008). Sex differences were not observed in the production of the pro-inflammatory cytokine TNFα or the anti-inflammatory cytokine IL-10 (Moldovan et al. 2008), and IL-17A production was not assessed. Of note, this Th1 bias in cytokine production in females was not observed in two other studies that examined sex differences in T-cell cytokine production in MS that used strong polyclonal stimuli (anti-CD3 and PMA/Ionomycin) rather than "weaker" antigenic peptides to elicit cytokine production (Nguyen et al. 2003; Eikelenboom et al. 2005). However, one of these studies did note a striking correlation between the frequency of IFN-γ-producing CD3^{+} cells in peripheral blood of MS patients and EDSS in females, but not in males (Nguyen et al. 2003), providing support for the notion that Th1-effector mechanisms may be more predominant in females with MS.

Consistent with the concept that sex differences in IFNγ production are dependent on the strength of the TCR stimulus, our group measured IFNγ production by male and female murine CD4^{+} T cells after stimulation with various concentrations of anti-CD3 and anti-CD28 and found that the sex difference in IFNγ production was only apparent when T cells were stimulated with submaximal doses of these stimuli (Dunn et al. 2007). We further observed in follow-up studies that we could observe a Th1 bias in cytokine production by female vs. male naïve T cells that were taken from the blood of healthy human volunteers upon stimulation with submaximal concentrations of anti-CD3 and anti-CD28 (Zhang et al. 2012). Interestingly, in this simple assay, male naive CD4^{+} T cells were instead biased toward the production of Th17 (not Th2) cytokines. A similar trend toward a Th1 bias in healthy women and of a Th17 or Th2 bias in healthy men has also been reported by one microarray study that compared gene expression between female and male PBMC after ex vivo activation (Hewagama et al. 2009).

Taken together, these studies provide compelling human and mouse evidence that autoreactive Th cells are biased toward Th1 cytokine production in females and toward Th17 and/or Th2 production in males. However, how these more robust Th1 responses are linked to a higher incidence of MS in women is not yet understood. The observation that female IL-17A-secreting Th cells that traffic to the spinal cords of female SJL mice during EAE are more likely than male Th cells to become "pathogenic" co-producers of IFNγ and IL-17A (Zhang et al. 2012) could offer one potential explanation for the higher encephalitogenicity of autoreactive T cells in MS women.

5.4 Sex Differences in BBB Permeability

A recent study by Cruz-Orengo et al. (2014) provided evidence that the female bias in EAE in SJL mice may also relate to sex differences in the expression of sphingosine-1-phosphate receptor 2 (S1PR2) and its role in regulating BBB permeability to lymphocytes (Cruz-Orengo et al. 2014). It was observed that female SJL mice exhibited higher expression of S1PR2 in certain brain regions as compared to males and that treatment of female, but not male mice with a specific S1PR2 antagonist reduced BBB permeability at various CNS sites and attenuated EAE severity (Cruz-Orengo et al. 2014). Furthermore, using an in vitro BBB culture system, it was demonstrated that S1PR2 signaling leads to dysregulated endothelial barrier functioning by activating Rho and CDC42, which signal the relocation of CXCL12 from the abluminal to the luminal side of BBB endothelium where this molecule is chemoattractive for lymphocytes (Cruz-Orengo et al. 2014). Thus, the differential expression of SIPR2 between the sexes may be another reason underlying the female bias in CNS autoimmunity.

5.5 Sex Differences in Microglia Number and Activation State

Once autoreactive T cells migrate into the CNS during EAE, microglia participate in the repriming of these cells and thus are critical to regulating disease incidence (Heppner et al. 2005). Microglia also actively mobilize to the sites of T-cell infiltration, proliferate, and contribute to inflammation by secretion of pro-inflammatory cytokines and nitric oxide (NO) (Goldmann and Prinz 2013). To date, the investigations of sex differences in microglial function have been very limited, but have suggested that this cell population does not account for sex differences in the development of CNS inflammation. For one, microglia numbers do not differ between the sexes in either the brains of adult mice (Manwani et al. 2013) or in biopsy samples of MS lesions (Kuhlmann et al. 2009). Furthermore, investigations of microglial function in adult rats have not detected any sex differences in the expression of pro-inflammatory mediators (TNFα, IL-6, IL-1, or inducible nitric oxide synthase, iNOS) by microglia, either in the steady state or after LPS stimulation (Sierra et al. 2007; Crain et al. 2013). In addition, Dasgupta et al. (2005) compared the gene expression of iNOS and NO production in cultures of primary male and female murine microglia that were co-cultured with either male or female myelin-reactive T-cell lines (Dasgupta et al. 2005). Though the authors noted that female T cells were more able than male T cells to elicit NO production by microglia, there were no differences observed in the production of this inflammatory mediator by male or female microglia when co-cultured with the same Th cells (Dasgupta et al. 2005). These findings further underscore the importance of the T cells in mediating sex differences in CNS inflammation during EAE.

5.6 Sex Differences in Humoral Immunity

Although MS is considered to be a T cell-mediated disease, B cells and autoantibodies are also thought to contribute to relapses and tissue damage in this disease (Krumbholz et al. 2012). Indeed, the most consistent immunological finding in MS is intrathecal immunoglobulin synthesis (Krumbholz et al. 2012), and the presence of antibodies and complement defines the most common lesion pattern (i.e., type II) found in MS (Lucchinetti et al. 2000). Furthermore, the finding that the B cell-depleting agent rituximab, had striking effects in decreasing relapse rate and inflammatory lesions in a recent phase II trial of RRMS (Hauser et al. 2008) has reinvigorated interest in B cells in MS.

Although sex differences in B-cell or antibody function have not been specifically investigated in either MS or EAE, there are clear indications from both human and mouse vaccination studies that females exhibit more robust humoral responses than males (Eidinger and Garrett 1972; Klein et al. 2010). Women display higher circulating levels of immunoglobulin than men in the steady state (Butterworth et al. 1967) and display more robust antibody responses to vaccination against a number of infectious agents as compared to men [reviewed in (Klein et al. 2010)]. Similarly, female mice have been shown to develop stronger and more lasting antibody responses to vaccination as compared to male mice to a variety of antigens (Eidinger and Garrett 1972). This greater humoral response is also thought to underly why autoantibody-driven disorders such as systemic lupus erythematosus predominate in women (Cohen-Solal et al. 2006).

While part of this greater humoral immune response in females is intimately linked to the more robust Th-cell responses in this sex, there is strong evidence that the female sex hormones estradiol and prolactin have direct actions on B cells to inhibit B-cell tolerance and to promote antibody production (Cohen-Solal et al. 2006) (see discussion of hormone action on B cells in Sect. 6). Whether sex differences exist in other B-cell properties such as APC function, cytokine production or B regulatory activity has not yet been investigated.

5.7 Summary

Together, past research suggests that women are more likely to develop MS because their adaptive immune responses are more robust than in males. Female myelin-reactive Th cells proliferate more and produce higher levels of Th1 cytokines upon encountering antigen in the context of a microbial infection and are better able to support humoral responses than male Th cells. In addition, recent data support the idea that the BBB in females may also be more permissive to the entry of autoreactive lymphocytes. On the other hand, there is no strong evidence to date that sex differences in Treg and microglia account for sex differences in MS initiation.

6 Role of Sex Hormones in the More Robust Autoimmunity in Females

The finding that there is a marked increase in both the incidence of MS and the female preponderance of this disease with pubertal onset suggests that hormonal changes that occur with puberty have a major influence on the biological mechanisms that are involved in MS initiation. In this section, we will overview what is known about the effects of gonadal (androgens and estrogens) and hypothalamic and pituitary sex hormones (GnRH and prolactin) on CNS autoimmune mechanisms.

6.1 Role of Androgens

It has been recognized for some time that the androgen receptor ligands, testosterone, and its metabolite dihydroxytestosterone (DHT) have suppressive effects on the development of CNS autoimmunity. Studies that manipulated the levels of these androgens in mice have demonstrated that sex differences in these levels account for the majority of the sex differences in Th immunity (Voskuhl and Palaszynski 2001). In EAE, castration leads to an increased severity of disease in males, and this increase correlates with a shift toward a more "feminine" profile of more robust T-cell expansion and Th1 cytokine production both in the periphery and CNS (Bebo et al. 1998; Zhang et al. 2012; Dunn et al. 2007; Voskuhl and Palaszynski 2001). On the other hand, treatment of females with testosterone or DHT inhibits the development of EAE in SJL mice post-vaccination with MBP and CFA (Dalal et al. 1997). Furthermore, growing myelin-reactive T cell lines in the presence of androgens inhibits the ability of these cells to transfer EAE (Bebo et al. 1999). In both active and adoptive transfer EAE, androgen-related protection correlates with a reduced production of IFNγ and higher IL-10 production by myelin-reactive $CD4^+$ T cells (Dalal et al. 1997; Bebo et al. 1999). Consistent with these results in mice, it was shown in a small trial in RRMS that daily testosterone treatment (100 mg/day, Androgel) suppressed delayed-type hypersensitivity responses to tetanus toxoid (a readout of Th1-mediated immunity), reduced the frequency of $CD4^+$ T cells in the blood, and decreased IL-2 production by PBMC (Gold et al. 2008).

The immunosuppressive effects of androgens occur through actions on macrophages and T cells, which both express the androgen receptor (Bebo et al. 1999). Treatment of female T cells with androgens results in a reduced ability to proliferate upon stimulation with anti-CD3 (Araneo et al. 1991; Liva and Voskuhl 2001). In addition, it has been shown that testosterone can reduce the production of pro-inflammatory cytokines (TNFα and IL-1β) by human macrophages (D'Agostino et al. 1999). Conversely, castration of male mice results in an enhanced proliferative capacity and IL-2 and IFNγ production by $CD4^+$ T cells (Dunn et al. 2007), and a shift toward a more pro-inflammatory macrophage profile (Wilcoxen et al. 2000).

Since most of these studies of androgen effects on immunity were conducted prior to the discovery of the Th17 subset, our group further investigated whether androgens are also driving the Th1/Th17 dichotomy observed between the sexes (Zhang et al. 2012). We found that in vivo treatment of female mouse T cells with DHT reduces the potential of these cells to produce IFNγ and increases the production of IL-17 upon ex vivo stimulation with anti-CD3 and anti-CD28 (Zhang et al. 2012). We found that this "reprogramming" did not occur when these studies were done in mice that were deficient in the nuclear receptor peroxisome proliferator-activated receptor-α (PPARα), thus implicating PPARα as an important mediator of androgen effects on Th1 differentiation in T cells (Zhang et al. 2012). Our follow-up studies further indicated that the differential Th1/Th17 cytokine production by female and male CD4^{+} T cells appeared to be controlled by both PPARα and the related nuclear receptor PPARγ. The mRNA expressions of both of these genes were found to be androgen sensitive in mice (Dunn et al. 2007) and in humans (Zhang et al. 2012) in that DHT treatment upregulated PPARα and suppressed PPARγ mRNAs in T cells (Zhang et al. 2012). Furthermore, PPARα was found to repress the Th1 pathway (Dunn et al. 2007; Jones et al. 2003), while PPARγ instead inhibited Th17 (Zhang et al. 2012; Kissick et al. 2014). Thus, one molecular mechanism of how androgens can modulate Th cytokine production appears to be through altering the ratio of these two nuclear receptors in T cells.

Recently, it was also discovered that androgens also can inhibit Th1 responses in mice by interfering with the phosphorylation of JAK2/TYK2 downstream of IL-12 receptor signaling (Kissick et al. 2014). Testosterone had these effects by inducing the expression of the phosphatase PTPN1 in T cells, which functions to dephosphorylate JAK2 and TYK2 (Kissick et al. 2014). It was further shown that the expression of PTPN1 was lowered in T cells that were taken from patients who were undergoing androgen deprivation therapy for the treatment of prostate cancer (Kissick et al. 2014), validating that this gene is androgen-sensitive in humans.

In regard to androgen levels in MS, a number of studies have measured testosterone in the circulation and found the level of this hormone to be lowered in both male and female MS patients (Tomassini et al. 2005; Foster et al. 2003; Bove et al. 2014). One study found that female patients with the lowest levels of testosterone were also the ones that exhibited the highest number of Gd-enhancing lesions, suggesting a relationship between active disease and the level of this hormone (Tomassini et al. 2005). However, it has been shown that encephalitogenic T-cell transfer has the effect of lowering the testosterone level in mice (Foster et al. 2003), suggesting that reductions in this hormone in MS may be both a consequence and cause of the inflammation that develops in this disease.

6.2 Role of Ovarian Hormones Estradiol and Progesterone

Despite the recently recognized role for female pubertal factors in increased MS risk, the prevailing notion based on studies in EAE is that the ovarian hormones estradiol

and progesterone are immunosuppressive and therefore do not contribute to the more robust Th immunity in females. However, the literature in this field indicates that the effect of estradiol in T cell- and B cell-mediated immunity is more enigmatic and that this hormone is both immunostimulatory and immunosuppressive in certain contexts. Thus, some previous research does support the potential involvement of the ovarian hormones in inflammation and early disease activity in MS.

The key evidence that ovarian hormones are immunosuppressive in EAE is that ovariectomy of adult female mice and rats results in increased EAE severity after active immunization (Matejuk et al. 2001; Jansson et al. 1994), while treatment of mice with hormone pellets that gradually release estradiol or progesterone protects against EAE development (Voskuhl and Palaszynski, 2001; Bebo et al. 2001; Garay et al. 2007; Yates et al. 2010). For progesterone, only the levels found during pregnancy or higher have been tested in EAE, and all were shown to attenuate disease (Garay et al. 2007; Yates et al. 2010). This protection was related to inhibition of the expansion and CNS infiltration of pro-inflammatory Th1/Th17 cells and to enhanced IL-10 production in the spleen (Yates et al. 2010). On the other hand, estradiol has been delivered to mice at doses that recapitulate diestrus (i.e., follicular phase in humans), metestrus (i.e., luteal phase in humans), or pregnancy levels, and all of these doses were shown to reduce EAE development in mice (Bebo et al. 2001). These immune-suppressive effects of estrogens on EAE are mediated through estrogen receptor alpha (Lelu et al. 2011) and correlate with (1) a reduced expansion and CNS infiltration of pro-inflammatory Th1 and Th17 cells (Lelu et al. 2011; Ito et al. 2001), (2) an increased frequency of FoxP3$^+$ Treg (Polanczyk et al. 2004), and (3) a shift of dendritic cells toward a more "tolerogenic" phenotype (characterized by higher PD-L1 expression and IL-10 production) (Polanczyk et al. 2006; Pettersson et al. 2004; Papenfuss et al. 2011). In a pilot study in RRMS, oral therapy with estriol, a form of estrogen prevalent in pregnancy, was shown to decrease TNFα and increase IL-5 production by circulating T cells and to increase IL-10 production by monocytes in women (Soldan et al. 2003).

Estradiol has also been shown to enhance T-cell adaptive immunity when administered at doses that recapitulate the lowest end of the physiological range (10^{-11}–10^{-9} M). For example, treatment of splenocytes in vitro with low-dose estradiol enhances IFNγ mRNA expression and IFNγ production induced by ConA or LPS stimulation (Nakaya et al. 2006; Fox et al. 1991), and this appears to occur through direct effects of estradiol on IFNγ promoter activity (Fox et al. 1991). Stimulatory effects of estrogens on dendritic cell maturation have also been noted, in that 10^{-10} M estradiol increases the yields of bone marrow-derived dendritic cells in culture and enhances the ability of these cells upon CD40 stimulation to prime responding T cells (Douin-Echinard et al. 2008). Finally, delivery of low levels of estradiol to female mice that were first ovariectomized was shown to enhance T-cell responses to vaccination relative to levels in ovariectomized placebo control counterparts (Maret et al. 2003). Together, these findings suggest that low levels of estrogens can enhance Th responses.

In contrast to the biphasic effects of estradiol on T-cell immunity, both low and high doses (i.e., pregnancy levels) of estradiol promote humoral immunity. Estradiol stimulates antibody production by PBMC (Kanda and Tamaki 1999) and has been shown to enhance circulating antibody levels in murine models of lupus (Roubinian et al. 1978; Peeva et al. 2000). Estradiol has been shown to stimulate humoral immunity by (1) promoting B-cell survival (through enhancement of BCL-2), (2) enhancing the threshold of B-cell activation, thus protecting B cells from apoptosis, and (3) promoting high-affinity IgG antibodies through deaminase-mediated class switching and somatic hypermutation [reviewed in (Cohen-Solal et al. 2006)]. The enhancing effects of pregnancy-level estrogens on B-cell auto-antibody production are in sharp contrast with the profound suppressive effects on T-cell immunity and are thought to be the reason why autoantibody disorders such as lupus flare with pregnancy (Ruiz-Irastorza et al. 1996), while relapse rates diminish in MS in the third trimester when estrogen levels are high (Confavreux et al. 1998).

6.3 Role of Hypothalamic and Pituitary-Derived Hormones

The levels of gonadotrophin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) do not differ between post-pubertal male and females (Neely et al. 1995) and thus are not likely responsible for the sex differences in Th immunity observed in adult men and women. However, these hormones do warrant mention since they undergo striking increases with pubertal onset (Grumbach 2002), are immunostimulatory in the context of MS (Correale et al. 2012), and thus may explain why MS risk increases post-puberty. With pubertal onset, the amplitude of GnRH pulses from hypothalamic neurons increases and triggers a higher pulsatile secretion of LH and FSH from the anterior pituitary (Grumbach 2002). Recently, the effects of GnRH on MS were highlighted by the findings that GnRH agonist treatment, when administered as part of assisted reproduction technology, had potent effects in increasing the risk of relapse (by sevenfold) and MRI disease activity (by ninefold) in a small group of RRMS patients (Correale et al. 2012). The increased disease activity was associated with an increased expansion and cytokine production by MOG- and MBP-reactive T cells in peripheral blood of these patients. Furthermore, this effect could be recapitulated by treating myelin-reactive T cells ex vivo with GnRH (Correale et al. 2012). Similar stimulatory effects of GnRH and GnRH agonists have been reported by other groups in rodent models and in human studies (Goldberg et al. 2009; Grasso et al. 1998). For instance, the treatment of mice with the GnRH agonist, lupron, has been shown to enhance T-cell reconstitution following lethal irradiation and allogeneic bone marrow transplant (Goldberg et al. 2009). Injection of humans with a bolus of GnRH was shown to enhance IFNγ levels in the serum and IFNγ production by PBMC stimulated ex vivo with ConA (Grasso et al. 1998). In addition, pituitary hormones, LH and FSH, have been shown to have modest effects in stimulating proliferation and cytokine production by human T cells in response to

stimulation with anti-CD3 and anti-CD28 (Carbone et al. 2010). Thus enhanced GnRH secretion could be one factor that is responsible for the increased incidence of MS observed post-puberty.

Prolactin is another pituitary-derived hormone that is elevated in the circulation during puberty and unlike GnRH is present at higher (twofold) levels in females than males (Roelfsema et al. 2012). The production of prolactin in the steady state is negatively regulated by dopaminergic inputs from the hypothalamus, while it is enhanced by thyroid-stimulating hormone, certain pro-inflammatory cytokines (IL-1, IL-6), stress, and the suckling reflex associated with breast-feeding (Chikanza 1999; Freeman et al. 2000). Although it has been speculated that the prolactin surge with breast-feeding is responsible for the increase in MS relapse activity observed in MS patients postpartum (Vukusic et al. 2004), this idea has been brought into question by recent findings that exclusive breast-feeding protects against post-partum relapses in MS (Langer-Gould et al. 2009). Nonetheless, there is a significant body of literature indicating that patients with MS and other autoimmune conditions exhibit higher levels of circulating prolactin and that this hormone has enhancing effects on the immune system in these diseases [for review see (Shelly et al. 2012)].

In MS, it is reported that 21–34 % of patients display prolactin levels above the normal range (Zhornitsky et al. 2013), which contrasts with the 0.5–3 % observed in the general population (Shelly et al. 2012). The underlying reasons for the higher levels of prolactin in MS and other autoimmune diseases are not known; however, it has been speculated to be due to effects of inflammation on the hypothalamic pituitary axis (Zhornitsky et al. 2013). Regarding its immunostimulatory effects, prolactin has been shown to enhance both T-cell and humoral immunity. This hormone has been shown to (1) enhance IFNγ production by ConA-stimulated PBMC (Chavez-Rueda et al. 2005), (2) increase T-bet expression by primary mouse $CD4^+$ T cells (Tomio et al. 2008), and (3) promote the GM-CSF-dependent maturation of dendritic cells from human monocytes (Matera et al. 2001). Additionally, in the Lewis rat EAE model induced by spinal cord homogenate and CFA, prolactin levels were elevated in the serum by 4-day post-immunization and pretreatment of rats with bromocristine, a dopaminergic agonist that tonically inhibits prolactin secretion, profoundly reduced EAE severity (Riskind et al. 1991). More recently, it was shown that deficiency in prolactin or the prolactin receptor in mice also results in a slight delay in T-cell trafficking and EAE onset in the C57BL6 model induced by MOG_{35-55} in CFA (Costanza et al. 2013).

In addition to these effects on T cell-mediated autoimmunity, prolactin is reported to have stimulatory effects on B cells (Correale et al. 2014). Prolactin has potent effects in stimulating MOG antibody production by MS patient PBMC in culture (Correale et al. 2014). Furthermore, those MS patients that have higher circulating prolactin levels also display a higher survival of B cells ex vivo, possibly due to prolactin effects in increasing the production of the B-cell survival factor BAFF and

BCL-2 expression (Correale et al. 2014). Furthermore, prolactin decreases the threshold of B-cell activation upon stimulation with anti-IgM and reduces CD40 expression on B cells isolated from MS patients (Correale et al. 2014).

6.4 Summary

The current evidence supports that notion that the higher testosterone levels in males coupled with the enhanced estradiol and prolactin levels observed in females post-puberty are potential drivers of the higher female-to-male sex ratio in MS observed post-puberty. Furthermore, increases in the level of gonadotrophins and/or prolactin with puberty may contribute to the rise in autoimmunity in MS observed in adolescence.

7 Underlying Role for Sex Chromosomes in the Female Preponderance of MS or EAE

Although a strong parent-of-origin effect has been identified in MS, where genetic susceptibility loci appear to be preferentially transmitted from mother to offspring (Ebers et al. 2004), a comprehensive scrutiny of candidate X-linked loci using a large MS familial database failed to find significant linkage between X loci and MS (Herrera et al. 2008). In addition, though skewed X inactivation, a process where cells preferentially express genes from either the maternal or paternal X chromosome, contributes to other autoimmune diseases such as thyroid disease and scleroderma, it does not appear to be a factor in MS (Knudsen et al. 2007). These findings, taken together with the fact that the female preponderance of disease is present post-, but not prepuberty, suggest that X- or Y-encoded genes are not a major driving force in the sex disparity in MS incidence.

Despite the lack of association found in MS studies, a number of investigations in genetic mouse models have provided evidence that X or Y chromosome-encoded genes play a role in EAE susceptibility in SJL mice. First, a study by Rhonda Vos-kuhl's group employed the "four-core genotype model" to parcel out the effect of the sex chromosome complement from gonadal hormones on myelin-specific responses in EAE (Smith-Bouvier et al. 2008). In this model, the testes-determining factor that normally resides on the Y chromosome (Sry) was moved to an autosome allowing the creation of gonadal males with both XY and XX (Sry transgenic) chromosome complements and gonadal females with XX and XY ($Sry^{-/-}$) chromosome complements (Smith-Bouvier et al. 2008). When hormone influences were removed by gonadectomy, the effect of the sex chromosome complement on autoimmune development could be parceled out (Smith-Bouvier et al. 2008). It was found through both active and adoptive transfer EAE studies in SJL mice that the XX

chromosome complement had a positive effect on disease development as compared with the XY chromosome complement (Smith-Bouvier et al. 2008).

Another genetic model that has called attention to the role of sex chromosome complement on EAE is the use of consomic mice that carry autosomes and one X chromosome from C57BL6/J, but have a Y chromosome derived from another strain (Case et al. 2013). Using this model, it was found consomic mice that carry a Y chromosome from SJL showed decreased EAE severity as compared to native C57BL6/J mice or consomic mice that carry a Y chromosome from alternative mouse strains (Case et al. 2013). The presence of the Y^{SJL} appeared to impact EAE susceptibility by influencing the transcriptome of macrophages and CD4$^+$ T cells (Case et al. 2013). While these studies clearly suggest a modulatory effect of sex chromosome complement on EAE development in SJL mouse, their relevance to MS is still unclear.

8 Conclusions and Future Directions

In conclusion, there is strong evidence that females are more susceptible to develop MS than men and that the autoimmune mechanisms that dominate in the early phase of this disease are more robust in females. Certain rodent models including EAE induction in the SJL mouse have been useful in the study of the biological basis of the sex dichotomy in CNS autoimmunity, and much progress has also been made into understanding the underlying immune mechanisms of the enhanced female susceptibility to autoimmunity. It has become clear that a more robust Th1 cell expansion contributes to the enhanced EAE susceptibility in female mice and that this sex difference in Th1 immunity is driven by higher expression of a number of androgen-sensitive genes in male T cells including PPARα and PTPN1. In addition, previous research has shown that female sex hormones may also contribute to the female bias of MS, particularly through enhancement of humoral immune responses. Further studies should be conducted in murine MS models where B cell- and autoantibody-dependent responses are known to contribute to disease pathogenesis (i.e., EAE induced in mice with whole myelin oligodendrocyte glycoprotein) in order to investigate the hormone–gene interactions that are involved in regulating sex differences in B-cell functioning. Future research should also evaluate sex differences in the activity of other immune cell types, particularly those involved in innate immune responses (macrophage, microglia, astrocytes, granulocytes, natural killer cells, natural killer T cells, or gamma delta T cells) as the role of these cells in mediating sex differences in CNS autoimmunity has not yet been adequately addressed either in MS or in murine MS models.

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