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Gender and sex hormones in multiple sclerosis pathology and therapy

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Abstract

Several lines of evidence indicate that gender affects the susceptibility and course of multiple sclerosis (MS) with a higher disease prevalence and overall better prognosis in women than men. This sex dimorphism may be explained by sex chromosome effects and effects of sex steroid hormones on the immune system, blood brain barrier or parenchymal central nervous system (CNS) cells. The well known improvement in disease during late pregnancy has also been linked to hormonal changes and has stimulated recent clinical studies to determine the efficacy of and tolerance to sex steroid therapeutic approaches. Both clinical and experimental studies indicate that sex steroid supplementation may be beneficial for MS. This could be related to anti-inflammatory actions on the immune system or CNS and to direct neuroprotective properties. Here, clinical and experimental data are reviewed with respect to the effects of sex hormones or gender in the pathology or therapy of MS or its rodent disease models. The different cellular targets as well as some molecular mechanisms likely involved are discussed.

Author Keywords Estradiol ; Estriol ; Progesterone ; Testosterone ; Neuroinflammation ; EAE ; Experimental Allergic Encephalomyelitis ; TMEV ; Th1 ; Th2 ; Autoimmune disease ; Dendritic cell ; Treg ; Review

BRIEF OVERVIEW OF MS PATHOGENESIS AND ANIMAL MODELS OF MS

Multiple sclerosis is a severe disorder of the CNS characterized by chronic inflammation, myelin loss, gliosis, varying degrees of axonal and oligodendrocyte pathology and progressive neurological dysfunction. The reader is referred to the concise review on MS pathogenesis by Gold et al (1). The prevailing animal model for MS is experimental autoimmune (or formerly termed 'allergic') encephalomyelitis (EAE), which can be induced in a variety of species, including genetically susceptible rodent strains, upon immunization with spinal cord homogenates, myelin or specific myelin peptides in combination with adjuvant (active EAE), as well as by adoptive transfer of encephalopathogenic (specific for myelin components) $CD4^+$ T cells (passive EAE). Theiler's murine encephalomyelitis virus (TMEV)-induced demyelination is a rodent viral model to induce brain inflammation and injury of the CNS myelin sheath with immune parameters and histopathology similar to those of chronic progressive MS. TMEV causes chronic demyelination in the CNS white matter of susceptible mice, while resistant strains are able to completely clear the virus before developing the late chronic demyelinating phase (2).

Experimental data have underlined the importance of CD4+ T cell involvement and of the T helper type 1 (Th1) and now type 17 (Th17) patterns of cytokine secretion in mediating the autoimmune processes associated with the destruction of myelin. However, other cellular players involved in innate and/or adaptive immune responses also play a role in the early and progressive events of the immune reaction leading to inflammation and CNS damage, such as CD8+ cytotoxic T cells, autoreactive B cells, subsets of natural killer cells and mast cells (3, 4). Most recently, spontaneous autoimmunity resembling MS was obtained by transgenic expression of selfreactive T cell and B cell receptors (5).

Despite important advances in therapeutics, none of the current disease-modifying drugs have been found to significantly alter the long-term prognosis of the disease. An increasing number of experimental and clinical data indicate that sex hormones may have therapeutical value and that gender and gonadal hormonal status should be taken into account for a more sex-appropriate targeted therapeutical strategy.

INFLUENCE OF GENDER ON SUSCEPTIBILITY TO MS AND ITS ANIMAL MODELS

Epidemiology

MS occurs more commonly in females than males as in the case of several autoimmune diseases. The prevalence of the disease is much greater in women and tends to follow a different clinical course (relapsing-remitting MS) than the one in the affected male population more prone to progressive MS, with a poor prognosis (6, 7). The mechanisms accounting for this gender difference in MS are coming to light and are discussed in this review. Surprisingly, MS prevalence increased faster among women than men in the last decades, with a female-to-male ratio reaching 4-to-1 in Northern countries, according to recent epidemiological data in U.S. or Canada that consolidate estimates in Norwegian, U.S. and French cohorts (8–12). The causes of this widening gender gap are unknown and do not

seem to stem from diagnostic and ascertainment methods (12). These observations should encourage efforts to identify potential environmental factors or habits accounting for the increased disease susceptibility in women. The possible link with the worldwide increasing prevalence of obesity/overweight is discussed later.

EAE and TMEV-D studies

Gender differences in EAE susceptibility have been initially described in rats though great variability was observed between labs or within experiments (13–14). No sex differences were reported in mice in early work (15). Further extensive and well-controlled studies clearly indicated that gender differences in EAE susceptibility and severity occur in certain mouse strains, in particular the SJL mouse (16–19, 148). This has been specifically addressed in various mouse strains using different encephalitogenic epitopes (20). Generally, when a sex dimorphism is observed, females exhibit increased EAE incidence, severity and/or duration (see Table 1). Orchidectomized SJL mice develop an EAE form closer to the female profile while ovariectomy generally does not affect drastically the EAE course (17, 20, 21). Gender differences in disease induction are largely explained by the influence of endogenous testosterone on the early immune response (17, 22, 148). In contrast, a sex dimorphism at the expense of males occur in the TMEV-induced demyelinating model reflecting the fact that males have less efficient virus clearance compared to females, allowing a strong demyelinating Th1 response to be mounted (23, 24). After castration prior to virus injection, males are even more sensitive to TMEV-induced demyelinating disease (25). In the late (demyelinating) phase of the disease, SJL female mice show higher neurohistopathological scores than males in accordance with EAE data (26).

Immunogenetics of sex differences

The use of classic genetics and whole-genome screening in different strains of mice or rats has identified several genetic regions that contain quantitative trait loci (QTL) conferring susceptibility or severity in EAE or TMEV-D (27). Interestingly, some non-MHC gene linkages have been shown to be affected by gender. Across autosomal chromosomes, unique loci with gender-specific effects have been shown in mice to govern susceptibility to remitting/relapsing (eae12) and monophasic remitting/nonrelapsing (eae7 and eae13) EAE (28). Several candidate genes in these loci and other specific loci are currently under identification (27). Among those, interferon (IFN) gamma, TAC1 and beta-chemokine genes are likely involved in rodents as well as in humans (29–32). Additional complexity is introduced by parent-of-origin effects that might reflect testis-determining gene (Sry)-independent sex chromosome polymorphism, capable of modifying disease susceptibility in both male and female mice (33–35).

Taken together, experimental animal studies indicate that some genetic factors differentially determine susceptibility and the clinical course in female vs. male. Linkage analysis in genetic studies of MS may be more informative if parental transmission vs. gender were given additional weight. Geneticians are however faced with the dilemma of adding gender stratification for the determination of such sex-interacting factors (with relative modest effects in the context of a large number of different potential predisposing genes) while large cohorts of patients and controls are required to gain statistical significance. However, assuming that a large body of pathogenic mechanisms is shared by different species, candidate MS genes generated by rodent experimental models will certainly help to identify specific targets involved in the genetics of sexual dimorphism of this complex genetic disorder. Interestingly, estrogen receptor alpha gene polymorphism is associated with MS in Finnish and Japanese but not Italian populations (36–38).

Gender specific differences in the central nervous and immune systems

The nervous system is sexually dimorphic, with gender-specific anatomical differences affecting various behavioral, physiological and hormonal responses. Sexual differentiation of the CNS is driven early during development by genes on sex chromosomes and during the perinatal period under the influence of the natural estrogen, 17beta-estradiol (here referred as estradiol) resulting from testosterone aromatization (39, 40). While some adult patterns of sexual dimorphism are present at birth (41), gonadal hormones still remain important for maintaining brain sex-specific differences later in life (42, 43). The most striking evidence for CNS sexual dimorphism possibly related to MS gender difference is that women have less cerebral white matter, which comprises the myelinated connecting axons (44–46). Accordingly, the density of oligodendrocytes in corpus callosum, fornix, and spinal cord is 20–40% greater in male vs. female rodents (42). This has been linked to gender-specific differences in oligodendrocyte progenitor renewal and maturation (42, 47). These anatomical differences may render female brain more susceptible to myelin attack in MS.

Gender differences in the hypothalamic-pituitary-adrenal axis (HPA) are also recognized in animals (48) and humans (49). HPA responses are generally greater in males subjected to a psychological stressor compared to females (50, 51), but inversely when other ACTH inducers, such as opioid antagonist naloxone or restraint, are used (51–53). It has been shown that the impregnation of the brain by gonadal hormones during the perinatal period is important for shaping the HPA sexual dimorphism, which is maintained in the adult by gonadal steroid hormone levels (54). It is recognized that stress and HPA, through release of catecholamines and glucocorticoids, affect major immune functions such as antigen presentation, leukocyte proliferation and trafficking, secretion of cytokines and antibodies (55). Thus, it has been hypothesized that susceptibility to autoimmune disease may be related to an impaired responsiveness of the HPA axis. Indeed, there is a growing literature suggesting that stress may affect the risk of exacerbation in patients with MS (56). Gender differences

in chronic stress responses may thus contribute to the increased susceptibility of women to MS, but clear evidence is yet lacking. Attempts to address this difficult issue in rodents in different models of multiple sclerosis (EAE and TMEV-D) gave contradictory and complex answers (26, 57–59). Nethertheless, the influence of psychological stress (such as social isolation) with gender on disease has not been tested in these models. Besides, it is not known whether the glucocorticoid resistance (decrease in the immune system's capacity to respond to the anti-inflammatory actions of glucocorticoids) observed in relapsing-remitting MS patients is different between men and women (60). In male mice, psychosocial stress induces a state of steroid insensitivity in splenocytes (61). Further investigations of gender × social stress interactions on disease incidence or progression are required.

Finally, it is worth noting that the immune system itself is also powerfully modulated by gender, early in development and during the perinatal period when sex steroid hormones may permanently alter the developmental pattern of T-cell repertoire, e.g. by profoundly altering glucocorticoid receptor expression in the thymus which directs and coordinates T-cell maturation and differentiation (62 for review). Indeed, the immune system of adult males and females exhibits differences not only in anatomy or cytology (e.g. thymus size, immunoglobulin levels) but also in its responsiveness. For example, females have a greater resistance to tolerance induction in some animal models as well as more pronounced tumor allograft rejection; accordingly, women compared to men have reduced antibody-dependent cell-mediated and natural killer (NK) cell cytotoxicity (63, 64 for reviews). As stressed before, several immune gene candidates are believed to be under the strong influence of gender and sex hormones. Recent evidence indicates that the control of regulatory T cell development is one of the key immune components of this sexual dimorphism (see below). The contribution of gender and gonadal hormones to the development of MS has received a lot of attention and will be discussed in detail.

HORMONAL FLUCTUATIONS AND DISEASE ACTIVITY

Ovarian cycle and menopause

Whether hormonal fluctuations in menstrual cycles or menopause are associated with exacerbations of MS symptoms has been difficult to address with questionnaire-based studies from retrospective studies with low number of patients (65–68). Though about 50% of patients reported worsening of MS symptoms at menopause, there are not yet definitive conclusions due to the low number of patients involved and the difficulty to differentiate between the subjective worsening as a consequence of hormonal changes and the natural progression of the disease which often occurs at that age (relapsing-remitting MS converting into a more progressive pathology). Moreover, serial magnetic resonance imaging (MRI)-based examinations were unable to show differences in brain lesion activity during ovarian cycle (69). Analysis of the ratio of progesterone/estradiol levels with the number and volume of gadolinium enhancing lesions gave conflicting results during the clinical course of relapsing-remitting MS (70, 71). However, conventional MRI, which detects brain white matter lesions and blood brain barrier disruption, is now considered as a poor indicator of severity and long-term progression of the clinical manifestations of MS (72–74). More advanced imaging analysis of the brain but also of the spinal cord may allow better correlation (74–76).

Disease activity and sex steroid levels

Disease itself can affect the levels of sex steroid hormone levels, due to damage in hypothalamic regions, dysfunction of the hypothalamo-pituitary-gonadal axis, or altered metabolism. In male rats, reduced testosterone levels are observed during EAE, and correlates with clinical symptoms (77). Blunted testosterone levels were also noted during passive (acute) EAE in SJL male mice, while, in females, no change in estradiol levels was noted in this model (78). In females, a report indicated that marked irregular estrus cycles are observed during symptomatic disease in C57BL/6 mice with active EAE, suggesting that chronic disease influences the hormonal state of females as well (79). Women with MS have lower estradiol levels during the luteal phase and slightly, though significant, lower plasma testosterone concentrations than normal subjects (69, 80). More strikingly, the women having the lowest testosterone concentrations had more brain lesions detected by MRI (69). While men with relapsing-remitting or secondary progressive MS and healthy men had generally similar sex hormone levels (69), a subset of male MS patients had lower testosterone levels (80). Strikingly, higher estradiol levels in men with MS were associated with a greater degree of brain tissue damage revealed by the extent of T2 hyperintense and T1 hypointense lesions (69).

Pregnancy

Several lines of evidence indicate that pregnancy and particularly the late stages of pregnancy are clearly associated with decrease in clinical symptoms or relapse rate in MS and animal models. Pregnant guinea pigs, rabbits, Lewis rats, and mice challenged with encephalitogen are relatively protected against EAE during the second and the third (last) week of gestation (81–84). Pregnant women also are less likely to develop MS during this period (85). It is now well recognized that the disease manifestation is reduced in pregnant women with relapsing-remitting MS (86, 98). This occurs particularly during the third trimester when levels of estrogens (estradiol and estriol) and progesterone (see Table 2) are elevated up to about 20 times (87). This seems well correlated with a decrease in active white matter lesions detected by MRI (88). This clinical improvement is however followed by temporary rebound exacerbations at post-partum, when the hormone levels decline. Over a long period, pregnancy does not seem to influence the progression of disability in MS (86).

Placenta-derived hormones are likely to account for pregnancy-related alterations such as a shift from Th1 to Th2 immune response, expansion of suppressive regulatory T lymphocytes and decrease in the number of circulating CD16+ natural killer (NK)-cells (89–91). Th1 lymphocytes secrete proinflammatory cytokines (e.g. IL-2, IFNgamma, lymphotoxin) while Th2 cells secrete anti-inflammatory cytokines (e.g. IL-4, IL-5, IL-10), which favor humoral-mediated responses. Importantly, Th2 cytokines are associated with down-regulation of Th1 cytokines and this Th2 shift is believed to provide protection from allograft rejection during pregnancy as well as from Th1-mediated autoimmune disease (92). Sex steroid hormones are likely the most important players underlying the mechanisms for diminished disease activity during pregnancy as they modulate various aspects of the immune response and brain homeostasis (see below). However, it is worth noting that the levels of other hormones with anti-inflammatory activity (1,25-dihydroxy-vitamin D_3, norepinephrine, cortisol) also increase by 2 to 4 times during late pregnancy (87, 93) and may contribute to the decrease in relapse rate during this time period. In particular, several lines of evidence suggest that vitamin D is an environmental factor affecting autoimmune disease prevalence and that 1,25-dihydroxy vitamin D_3 induces regulatory T-cell function important for development of self-tolerance (94, 95).

The high circulating concentrations of estrogen during late pregnancy also promote prolactin secretion. Prolactin is produced not only by the anterior pituitary but also by extra-pituitary tissues such as the endometrium and the immune system where it can increase the expression of co-stimulatory molecules or cytokine secretions from T cells, B cells, NK cells and dendritic cells (96). As for sex steroid hormones, prolactin levels fall after birth but breast-feeding reestablishes prolactin secretion for milk production. However, breast-feeding does not alter the relapse rate in women with MS (97, 98). This suggests that circulating prolactin itself does not play a major role in reducing disease activity in relapse-remitting MS. A slight increase in prolactin levels in premenopausal women with MS has been reported (99–101). This has not been yet confirmed in a large cohort and may be secondary to hypothalamic lesions. Interestingly, it has been recently demonstrated that prolactin promotes oligodendrocyte precursor proliferation and stimulate myelin repair in mice (102). Thus, it is tempting to hypothesize that prolactin may reduce the disease progression or severity - but not relapse rate - by protecting myelin, a possibility that requires further investigations while already offering a new therapeutical strategy.

Other factors found during pregnancy also exhibit anti-inflammatory properties such as pregnancy-specific glycoproteins, alpha-fetoprotein, an estradiol-binding protein with immunoregulatory functions, Early Pregnancy Factor, and relaxin (103–107). The two latter are found at higher levels in early pregnancy than close to the time of birth, and likely shift locally the immune response from Th1 to Th2 to protect the fetus from allorejection during implantation. These various pregnancy molecules have interesting anti-inflammatory or immunoregulatory properties (108–111). They may be useful to develop new MS therapeutical approaches. Interestingly, alpha-fetoprotein decreased disease severity and various aspects of chronic EAE neuroinflammation including axonal pathology, T-cell reactivity, and antigen presentation in mice (109).

The potential role of leptin

Another endocrine factor which may underlie the sex differences in MS/EAE vulnerability is leptin as its serum levels are about three times higher in females than in males and remain higher even after adjusting for body fat (112–114). Sex steroid hormones, in particular testosterone, are significant determinants of the sex difference in serum leptin levels (115). Leptin is a pleiotropic hormone produced primarily by adipocytes but also by T lymphocytes and neurons (116, 117). Several lines of evidence indicate that leptin contributes to EAE/MS pathogenesis, influencing its onset and clinical severity, by acting as a proinflammatory cytokine which promotes regulatory T cell (Treg) anergy and hyporesponsiveness, resulting in increased Th1 (TNFalpha, INFgamma) and reduced Th2 (IL-4) cytokine production (116–120). Accordingly, circulating leptin levels are increased in relapsing-remitting MS patients (men and women analyzed together) while the CD4+CD25+Treg population decreases (119). As the leptin plasma concentrations are proportional to the amount of fat tissue, obese/overweight individuals produce higher levels of leptin. Whether the increasing MS prevalence in women vs. men as mentioned earlier is linked to the worldwide increasing prevalence of obesity and an enhanced immune sensitivity of women to leptin remains to be examined.

In conclusion, several endocrine factors in concert are likely to contribute to MS sexual dimorphism and protection during pregnancy. While cytokines and pregnancy-specific factors are undoubtly important in mediating protection, sex steroid hormones might play a critical role in the control of autoimmune diseases such as MS, by acting on various immune and non-immune/neural systems as indicated below.

EFFECTS OF EXOGENOUS SEX STEROIDS IN MS AND EAE

Oral contraceptives and MS

The numerous studies on the influence of birth control pills (containing estrogens and progestagens) on health have given clinicians the opportunity to assess the impact of oral contraceptives on MS incidence. Until recently, it was believed that oral contraceptives do not affect the risk of developing MS. A large prospective study from two cohorts in USA, the Nurse's Health Study (NHS, 1976–1994) and the Nurse's Health Study II (NHSII, 1989–1995) performed by Harvard School of Public Health and coll. did not support a lasting protective effect on MS incidence, in accordance with previous studies issued from smaller British cohorts (121–123). In the NHS's studies, the

analysis was restricted to women who did or did not use oral contraceptive 4 years before MS diagnosis to ensure that women did not change their contraceptive behavior after the occurrence of the initial neurological symptoms. Thus, the effect of recently taken steroids could not be assessed. A recent case-control study performed on the large British General Practice Research Database analyzed MS incidence in women with at least 3 years of continuous information before the date of first symptoms; the incidence of MS was in fact 40% lower in recent users of oral contraceptives (mainly ethinyl estradiol plus a progestagen) compared with nonusers (124). Another study suggested that oral contraceptive use is associated with decreased severity of MS symptoms (125). Taken together, these observations indicate that oral contraceptives are rather beneficial for MS patients.

Estrogens and EAE

In rodents, several lines of evidence have now indicated that estrogens (mainly given as subcutaneous implants of estradiol or estriol), even at low doses equivalent to diestrus/estrus levels, delay EAE onset and reduce or suppress disease activity when the treatment starts before disease induction in both males and females (126, see Table 3). This has been associated with a reduced leukocyte infiltration and altered production of proinflammatory cytokines including TNFalpha but, generally, with only a slight increase in Th2 cytokine production such as IL-4, IL-5 or IL-10 from activated spleen or CNS mononuclear cells cultured from estrogen pretreated and immunized mice (127–132, see section 6.2). Not surprisingly, CNS cytokine expression and neurohistopathological markers in estrogen protected EAE mice are similar to control healthy mice (128, 129, 132, see Table 3). It is interesting to note that the minimal effective dose that inhibits EAE varies greatly between mouse strains (126). This may indicate that estrogen receptor sensitivity may be a key issue in MS prevalence in some individuals or populations.

Estrogens bind to two classically known estrogen receptors (ER), ERalpha and ERbeta. Recent data indicates that ERalpha, but not ERbeta, is crucial for the protective effect of estrogens in EAE (133–135). While lymphocytes clearly express estrogen receptors and their response can be modulated by sex steroids (section 6.2), experiments using transfer of ER deficient vs. wild type effector T cells did not show differences in EAE; rather, ERalpha-expressing non-lymphocytic cells are required for this protective effect (136–138). Estrogens likely play a protective role through their pleiotropic effects on antigen presenting cells, endothelial cells as well as on the different brain cell types by down-regulating the inflammatory response and by its direct neuroprotective properties through ER (ERalpha and ERbeta)-mediated genomic as well as ER-dependent or -independent membrane effects (see paragraph 6.1). Interestingly, B10.PL male mice with a disrupted ERalpha develop less EAE symptoms after the acute initial phase while ERbeta$^{-/wt}$ (but not ERbeta$^{-/-}$) male mice develop more severe disease, suggesting that endogenous estradiol (e.g. from testosterone aromatization) may also exert regulatory functions in males through ERalpha and ERbeta, the latter involving the nonhematopoietic compartment (139). The interpretation of ERalpha involvement in males must be taken with caution since increased plasma testosterone (and estradiol) levels are observed in ERalpha knockout males (140). ERbeta agonists have also been shown to exert substantial neuroprotective effects on late (active) EAE symptoms in female C57BL/6 mice (141). In contrast, no effect was found in a passive EAE mouse model (135).

Data concerning the therapeutic effect of estrogens in EAE models, treatment starting after disease onset, are less numerous and somehow not consistent. Studies agree that low levels of estrogens (estradiol or estriol), when given at onset of symptoms, are unable to affect disease progression in EAE models unlike the protective effect previously mentioned. Subcutaneous implants of estradiol to reproduce pregnancy levels did not significantly affect disease (126, 142). In contrast, ethinyl 17alpha-estradiol, an orally active synthetic estrogen, reduced clinical severity in SJL mice, even when given at the onset of symptoms (130). Treatment with late pregnancy doses of estriol was slightly effective in the passive EAE study by Kim et al (127). This may be due to the mixed agonist/antagonist property of estriol (see section 7) or its 3-4-fold higher affinity for ERbeta than for ERalpha (143).

Androgens and EAE

When evaluating the influence of testosterone supplementation in vivo, it should be considered that some effects of testosterone could be mediated via the ER pathway. First, testosterone can be converted into estradiol after action of aromatase, which is expressed by various cell types including adipocytes, brain cells and circulating leukocytes (144–145). Second, testosterone metabolites can directly activate ER. Indeed, testosterone is converted into the more potent androgen 5alpha-dihydrotestosterone (DHT) by 5-alpha reductase. This active androgen cannot be converted into estradiol by aromatase and has been used in experimental settings to ensure a role for an androgen receptor (AR)-mediated pathway. However, recent data indicate that 5alpha-androstane-3beta, 17beta-diol (3betaAdiol), a DHT metabolite, can act on estrogen receptor beta (146, 147). Thus, when examining testosterone or DHT effects, the potential action on ER should be kept in mind.

As already indicated, the sexual dimorphism in EAE has been mostly analyzed in SJL mice (16, 17, 148–150; see Table 1). In this strain, endogenous testosterone likely through AR is indeed protective mainly acting on the induction phase in contrast to the C57Bl/6 strain (22, 150). However, when very high numbers of encephalitogenic T cells obtained from female mice are used to induce passive EAE in SJL mice, the sex dimorphism associated with the clinical development of EAE is no longer observed (22). This sexual dimorphism has been linked to an androgen-mediated Th2 bias, as suggested by the INFgamma/IL-10 ratio in the supernatant of encephalogenic peptide-specific T cell clones (22, 148, 151, see section 6.2). Interestingly, implants of testosterone (leading to serum levels of ~ 30 ng/ml,

concentrations physiologically reached during social encounter in males) as well as DHT were found protective in all strains of (male or female) mice studied (21, 148–150, 152).

Progestagens and EAE

In rats, while ethinyl estradiol inhibited EAE, the progestagen medroxyprogesterone acetate (MPA) augmented disease activity (153). Similarly, progesterone-treated ovariectomized Lewis rats had more severe sensorimotor deficits with increased inflammatory infiltrates and, strikingly, increased neuronal apoptosis after active EAE induction with myelin basic protein, though coadministration of estradiol prevented these consequences (154). In contrast, progesterone treatment before or after the adoptive transfer of encephalopathic T cells did not affect the course or severity of EAE in SJL female mice (127). Treatment of rats with MPA alone after disease induction did not alter the course of EAE, however it was shown to potentiate the effectiveness of a corticosteroid agonist (155). The causes of the discrepancies in these studies are not resolved, but are likely due to the different disease induction procedures (active vs. passive EAE). Nethertheless, on the neuroprotective and anti-inflammatory perspective, they raise questions about the actual benefit of progesterone use in MS therapies (see section 7).

Pilot studies and current clinical trials

Based on the observations that patients with MS have fewer relapses during late pregnancy and on decades of experimental data showing the beneficial effects of estrogenic treatment on EAE development, a pilot clinical trial was performed by Sicotte et al (156). The study showed that estriol caused significant decreases in brain lesion activity in six relapsing remitting MS women. Though preliminary, this clinical data definitely led to consider sexual steroids as new potential therapeutic tools for MS and is now followed up on a larger scale with oral estriol in combination with subcutaneous glatimer acetate. Moreover, other clinical trials based on the classical hormone therapy of menopause, an estrogen combined with a progestin, are underway. The European POPART'MUS study has been designed for women with MS in their post-partum period (transdermal estradiol plus the progestative nomegestrol acetate given orally) (157). Another study will test the safety and tolerability of oral ethinylestradiol and the progestative desogestrel combined with Interferon-Beta-1a in relapsing-remitting MS female patients (Tomassini V, Marinelli F, Pozzilli C).

Recently, Sicotte et al tested testosterone gel treatment (increasing hormone levels by 2-fold) for one year on 10 male patients with relapsing-remitting MS and found improvements in spatial and working memory performance and a slowing of brain atrophy (158). No significant alteration in inflammatory activity as assessed by MRI was noticed. This contrasts with the estriol pilot study in women, though the number of subjects examined, the low basal level of MRI activity and the mildness of clinical symptoms may not yield definitive conclusions. These findings suggest however that testosterone treatment is well tolerated and has potential neuroprotective effects in men with relapsing-remitting MS. Moreover, sexual dysfunction can be observed in men with MS, in particular in patients showing hypothalamic lesions or third ventricle enlargement and lower testosterone levels. Androgen supplementation improved libido and erectile dysfunction in such patients (78).

EFFECTS OF SEX STEROID ON IMMUNE SYSTEM AND CNS

An outline of the various sex steroid receptor and cellular targets in relation to the inflammatory process and neuroprotection is necessary for considering how sex steroids might affect the onset or progression of multiple sclerosis.

Overview of sex steroid signaling

Estrogen

The two well known estrogen receptors ERalpha and ERbeta can act by regulating transcriptional processes (159). The classical mechanism of ER genomic action involves estrogen binding to receptors in the nucleus, after which the receptors dimerize and bind to specific response elements known as estrogen response elements (EREs) located in the promoters of target genes including cytokine encoding genes, e.g. IFNgamma which is induced by estradiol in lymphocytes in vitro (160). However, these ERs can also regulate gene expression without binding to DNA directly but via protein-protein interactions with DNA-binding transcription factors in the nucleus. This may account for the estrogenic regulation of promoter activity of several pro-inflammatory cytokines (161).

In addition, membrane-associated ERs also mediate nongenomic actions of estrogens (162), which can lead to regulation of gene expression through second messengers and modulation of protein kinase activities (163, 164). Estrogens can also have indirect effects due to modulation of cation fluxes including calcium (162). Moreover, as ERalpha or ERbeta are localized to the mitochondria, they may directly regulate the expression of ERE containing mitochondrial genes (165–169). Other estrogen receptors involved in the rapid signaling of estrogen have been recently described, such as the GPR30, a G protein-coupled estrogen receptor which is predominantly localized in the endoplasmic reticulum (170, 171). Another example is the ER-X which is enriched in caveolar-like microdomains of cellular membranes and also binds progesterone with less potency (172). The high expression of GPR30 transcripts in lymphoid cells and tissues suggests that the receptor may function in the regulation of the inflammatory system (173).

Estrogen receptor-independent antioxidant effects due to intrinsic free-radical scavenging properties of estrogens have also been proposed from in vitro studies as a potential mechanism by which the hormone may protect against several insults (174–176). However, recent experimental in vitro data do not support this mechanism (177). Moreover, the nonphysiological levels of estradiol (0.1 to 10 μM) required in vitro for this action are not achieved in vivo, even with supraphysiological plasma levels found during pregnancy. Therefore, direct antioxidant effect of estrogens is unlikely to be relevant in vivo (178). A local role cannot however be excluded in estradiol producing-cells where it could reach sufficient concentration, and block lipid peroxidation reactions by intercalating into mitochondrial cell membranes (179). As the biological importance of the direct antioxidant properties of estrogen is still controversial, it will not be discussed further. On the other hand, indirect estrogen receptor-dependant antioxidant effects can be mediated via mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NFkB) signaling pathways, resulting in an upregulation of antioxidant enzymes (180). Other mechanisms accounting for the beneficial effects of sex steroids are linked to their immunoregulatory, anti-inflammatory and direct neuroprotective properties which are described below. Only «physiological» levels of steroids, below 0.1 μM for estrogens or androgens and below 1 μM for progesterone, will be considered (see Table 2).

Progesterone

As in the case of estrogen, progesterone can have membrane receptor-mediated effects and also modulate genomic pathways via the nuclear progesterone receptor (PR). Two main isoforms of this PR have been described: PR-B and the shorter form lacking 164 amino acids at the N-terminus, PR-A that is a weak transcriptional activator of specific target genes and a strong repressor of transactivation by PRB and other steroid receptors (181). Besides, new membrane progesterone receptors and progesterone binding proteins, unrelated to PR-A/PR-B, have been recently discovered but their role in neuroinflammation or neuroprotection have been poorly explored. Progesterone can also be converted into allopregnanolone, in vivo, which modulates the GABA-A receptor, an action accounting for some of the rapid membrane effects attributed to progesterone. The reader is directed to the review by Schumacher et al that provides an insightful perspective on the pharmacology and neuroprotective and clinical consequences of progesterone signaling (182).

Androgen

Similar to estrogen, androgens, including testosterone and the more active metabolite DHT, are known to exert their effects through the activation of intracellular receptors that regulate the transcription of target genes. Two isoforms of the classical AR have been described (AR-B and its N-terminally truncated form, AR-A) and are expressed in many different cell types (181). As for estrogen or progesterone, the existence of a plasma membrane receptor for androgens has also been proposed; classical genomic and nongenomic mechanisms, including the activation of signaling pathways as the MAPK pathways, have been described in neuronal cell line (PC12) and glial cells (183–186).

Immune system

The correlations between sex hormone levels and the activity of the cytokine-secreting immune cells from rodents as well as humans have lead to the idea that sex hormones directly influence the cytokine milieu in the immune system (reviewed extensively by others, 187–189).

Estrogens at levels far below pregnancy and progesterone have been shown to have stimulatory effects on the immune system, especially on B cells. Low levels of estrogens favor a proinflammatory Th1 response, whereas progesterone and high doses of estrogens favor a Th2 response by upregulating the production of IL-4 or IL-10 and down regulating TNFalpha secretion from immune cells. Testosterone is considered immunosuppressive regarding T and B cell activation in rodents as well as humans (187, 190, 191). More complex immunomodulatory effects are now reported and highly depend on the immune cell activation context and disease status (189). Experiments showing that estradiol at 10–100 nM inhibits lipopolysaccharide (LPS)-induced TNFalpha production from human peripheral blood mononuclear cells (PBMCs) but is stimulatory in the absence of LPS illustrate the importance of cellular context (192). In fact, sex hormones exert pleiotropic effects, depending upon concentration (in particular for estrogens), their conversion in other metabolites and their interaction with the local milieu at multiple levels, affecting lymphohematopoietic cell development, proliferation, apoptosis, activation and cytokine or antibody production. The net effects of sex steroids on the complex interactions between immune cells and the local milieu drive the final outcome, increasing or dampening the autoimmune pathological response. Though estrogens have been shown to have a direct stimulatory effect on IFNgamma lymphocytic gene expression in vitro, ovariectomy up-regulates IFNgamma production by Th cells from bone marrow and secondary lymphoid organs in mice (160, 193). As INFgamma stimulates macrophages to express IL-12 and IL-18 and the major histocompatibility complex class II, it can lead to increased antigen presentation to T cells and production of INFgamma and TNFalpha by T cells (193, 194). Taken together, these data suggest that, in vivo, female sex hormones during ovarian cycle are able to dampen the activation of the immune system.

Testosterone, likely after local enzymatic conversion into estradiol, and estrogens at pregnancy levels also enhance suppressor T cell activity, which may be explained by preservation and amplification of the suppressive CD4+ CD25+ Treg cell population and/or alteration in the number and activity of natural killer (NK), natural killer T (NKT), or invariant natural killer T (iNKT) cells, other important immune

regulators in multiple sclerosis (89, 195–204). These actions may be direct on NKT and iNKT or via interaction with antigen presenting cells such as dentritic cells (DCs) which are also sensitive to sex steroid action (201, 205–207).

Strikingly, altered mRNA expression of estrogen and androgen receptors were recently noted in peripheral mononuclear cells isolated from MS patients from Sassari as compared to healthy controls, indicating that in some human populations altered expression of sex steroid receptors in leukocytes (or other cell types) may contribute to MS pathology (208).

T cells and NK cells

Though initially difficult to detect by immunocytochemistry or binding studies from cytosol or nuclear extracts, the presence of estrogen receptors ERalpha and ERbeta by RT-PCR in rodent thymic or peripheral lymphocytes (209) indicated that estrogen may directly affect immune cells during their development and mature function. Similarly, human peripheral blood CD4+T cells have been shown to express relative high levels of ERalpha compared to ERbeta while peripheral blood CD8+ T cells and monocytes express low levels of ERalpha and ERbeta; these different cell populations did not exhibit sex differences in ERalpha/beta expression (210). Of note, ERalpha is expressed by CD4+CD25− T cells and its activation favors the conversion into CD4+CD25+ regulatory T cells (197). Adult bone marrow lymphocyte precursors (but not liver/embryonic precursors) also express ER and AR (211). CD4+ and CD8+ T lymphocytes have been shown to express the AR mRNA as well (151). The localization and functioning of AR is tissue specific: in thymic T cells, AR is expressed intracellularly, but not at the membrane, and mediate the nuclear androgen action; in contrast, splenic T cells express functionally active AR at the membrane, whereas their expressed intracellular ARs are not functional in the genomic pathway (212, 213). The presence of PR on lymphocytes remains controversial and may be only detectable levels during pregnancy (191, 214, 215). The expression of PR and AR in thymic stromal/epithelial cells is also important for the sex steroid-mediated control of thymic size and thymocyte development (216, 217). Importantly, estrogens also act at the transcriptional level to modulate $beta_2$-adrenergic receptor expression and coupling during maturation of the thymus, with consequent alterations on T-cell mediated immune responses (62).

Even before the precise confirmation of sex steroid receptor expression in T cells, sex hormones had been shown to selectively modify cytokine secretion from antigen specific T cell lines. The secretion of the main pro-inflammatory cytokines, IFNgamma and TNFalpha, has long been known to be influenced by gender in MS patients (218, 219). The Th1 skewing of immune responses in female patients represents a plausible mechanism for progression of disability, as increased IFNgamma production best correlated with disease severity in females but not in males (218, 219). This situation is temporarily relieved during late pregnancy when the IL-10/IFNgamma ratio increases, even in relapsing-remitting MS patients (220). This is in accordance with experimental data showing that high levels of estrogens and progesterone favor the Th2 immune response, at the expense of Th1 cytokine production and cell-mediated immunity. In human antigen specific CD4+ T cell clones obtained from multiple sclerosis patients, estrogen (estradiol or estriol) only at pregnancy levels enhanced secretion of antigen- or anti-CD3-stimulated IL-10 and IFNgamma (221, 222). In contrast, estrogens had a biphasic effect on TNFalpha secretion, with concentrations below 10 nM being stimulatory, and above 20 nM, concentrations reached at late pregnancy, being inhibitory. None of the estrogens influenced IL-4 or TGFbeta secretion while progesterone at late pregnancy levels (>30 nM) enhanced secretion of IL-4 from antigen-specific human CD4+ T cell clones (222, 223). In another study, similar changes in IL-10 and TNFalpha levels (but not INFgamma) were obtained after treatment of human T cell clones with high levels (>20 nM) of estriol, which also inhibited T cell migration and was associated with inhibition of NFkB signaling (111). A partial Th1 to Th2 shift was also observed in stimulated PBMCs from relapsing-remitting MS patients which had received estriol supplementation, as assessed by slight increased production of IL-5, primarily by CD4+ and CD8+ T cells, and IL-10, mostly by CD64+ monocytes/macrophages, and decreased TNFalpha, primarily by CD8+ T cells. In contrast, cytokine production by B cells was unaffected (224). These modest changes (10–20%) in cytokine profiles were correlated with the mean volume of enhancing lesions on MRI (224). The fact that exogenous estrogen is still able to prevent EAE development in IL-4 knockout and IL-10 knockout mice suggest however that these Th2 cytokines are dispensable for the estrogen protective effect in mice, though it may be restricted to the B6.129 strain background (128).

These studies from human PMBCs indicate that the female immune system, which is prone to Th1 skewing as compared to males, can be slightly directed towards a Th2-like cytokine profile during high estrogen exposure. Several experimental studies in rodents confirmed that sex steroid dosage is determinant in these alterations but also extended the notion that other factors must be taken into account: recruitment and CNS vs. peripheral T cell behavior. In vivo, treatment of ovariectomized mice with low/estrus levels of estradiol has been shown to rather enhance non CNS antigen-specific CD4+ T cell responses from draining lymph node cells (INFgamma secretion and CD4 + T cell proliferation) suggesting increased Th1 development, an effect that required functional expression of ERalpha but not ERbeta in bone marrow derived cells (225). However, the absolute number of CD4+ and CD8+ T lymphocytes in secondary lymphoid organs was decreased in these mice suggesting a diminished recruitment of T cells or reduced lymphopoiesis. In mice with EAE, pretreated with high concentrations of estrogen, a similar increase in INFgamma response and a trend for increased TNFalpha response are found in spleen T cells (130). Others found modest changes in Th1/Th2 cytokine profiles, with increased IL-10 production by T cells and/or macrophages and rather slightly decreased INFgamma production from draining lymph node cells or splenocytes derived from treated animals (142). In contrast, pregnant (C57BL/6) mice show a clear skewing of activated spleen T cell responses toward Th2, as indicated by decreased production of INFgamma or TNFalpha and increased expression of IL-4 or IL-10 (89). This is consistent with initial observations showing

that serum from rats treated with high dose of estradiol over two weeks caused decreased T lymphocyte response and enhanced B lymphocyte activity (226). Strikingly, mononuclear cells isolated from CNS of EAE mice displayed a very different pattern of cytokine production as compared to spleen cells, as reflected by their marked decrease in INFgamma and TNFalpha responses in estrogen versus vehicle pretreated mice with no increase in the Th2 cytokine profiles (130). Moreover, less T cells showing a decreased proportion of TNFalpha or INFgamma producing CD4+ cell subpopulation are recovered from the CNS of estrogen pretreated as compared to vehicle pretreated EAE mice (129, 130). Therefore, it is possible that this decreased Th1 pattern of CNS mononuclear cells in estrogen pre-treated mice reflects the local suppression or anergy of encephalopathic CD4+ cells. The fact that the regulation of T cells infiltrating the CNS differs drastically from the peripheral lymphoid cell pool is puzzling and needs further investigations.

Besides, the rather modest changes in Th2 cytokine secretion and proliferation responses to encephalitogen peptides in mixed lymphocyte reaction assays using immune cells from estrogen treated animals is partly explained by the fact that estrogen also directly alters the stimulatory activity of antigen presenting cells and the suppressive activity of Tregs as discussed later (89). Moreover, an increased INFgamma and TNFalpha secretion is not an obligatory indicator of a skewed immune response leading to tissue damage. First, a subpopulation of CD8+ T cells (CD8+ Tregs) can secrete INFgamma with IL-10 (227). Second, INFgamma and TNFalpha can down-regulate cytotoxic CD8+ T cell responses by inducing apoptosis (228–230). Indeed, despite beneficial effects in EAE mice, a randomized placebo-controlled study demonstrated that TNFalpha blockade rather worsened disease in patients with MS (231). Finally, INFgamma can also interact in concert with other cytokines such IL-27 or IL-4 to mediate anti-inflammatory brake functions, involving the regulation of the new Th17 subset (232).

A Th1-to-Th2 immune shift is a more plausible mechanism to account for the beneficial effect of androgen signaling in EAE and MS. Indeed, exogenous administration of testosterone or DHT in male or female mice decreases EAE severity by directly promoting the production of IL-10 at the expense of IFNgamma from myelin reactive CD4+ lymphocytes (22, 148, 150, 151). Dunn et al have shown that the expression of the peroxysome proliferator-activated receptor alpha (PPARalpha), belonging to the nuclear hormone receptor family and acting as a transcription factor to reduce inflammatory pathways in immune cells, is higher is male vs. female CD4+ T cells in SV.129, C57BL/6 and SJL mouse strains and is controlled by testosterone (233). Moreover, PPARalpha expression in T cells plays a key role in dampening the Th1 responses in males. This T lymphocyte intrinsic mechanism is unlikely to be sufficient to account for the sex differences in disease onset and progression as no sex dimorphism in EAE development is observed in SV129 and C57/B6 mice, suggesting that other factors or immune cells are involved. Nethertheless, these studies clearly support the concept that androgens shape the development of effector T cells via several mechanisms and play an important role in governing gender differences in the development of EAE/MS disease.

Differences in regulatory T (Treg) cell number or suppressive capacity are now believed to have a significant role in mediating sex differences and sex steroid effects in EAE/MS susceptibility or severity, as mentioned earlier. For example, in the resistant mouse strain BP10-S, inhibition of Treg cells with CD25 antibody renders male mice highly susceptible to EAE, while moderately predisposing female mice, and in vitro experimental data suggest that the expansion of pathogenic T cells by CD4+CD25+ Treg cells is more effective in males than females (196). Increased susceptibility is indeed associated with an enhanced effector T cell proliferation and greater production of IFNgamma, IL-6, and IL-17 (196). Peripheral CD4+CD25+ Treg cells from MS patients have reduced expression of Foxp3, which is involved in maintaining immune tolerance and preventing autoimmune diseases (234). Interestingly, estrogen at pregnancy levels expands Treg cell population, increases Foxp3 expression in mice through ERalpha and converts 15% of ER expressing CD4+CD25− T cells into CD4+CD25+ Treg cells after anti-CD3/CD28 activation in vitro (89, 195, 197). In line with these animal studies, a randomized trial in 12 healthy men indicates that medical castration significantly reduces the percentage of CD4+CD25+ T cells and decreases IFNgamma expression in mitogen-induced CD8+ T cells (200). Treg cells also expand during the follicular phase of the menstrual cycle, a process which seems requiring more than estradiol (235).

Thus, the potential increase in the activity that suppresses encephalogenic T cells likely contributes not only to sex differences in immune responses but also to the beneficial effect of testosterone and estrogen supplementation on EAE/MS development. As indicated earlier, studies using adoptive transfer of effector T lymphocytes derived from ERalpha or ERbeta knockout vs. wild type mice to induce EAE, suggest that estrogen signaling on encephalopathogenic CD4+ T cells is dispensable for inhibition of EAE by estrogen. However, this does not rule out a direct action on different lymphocyte populations. Gender differences and sex steroid effects on the immune response may be mediated by complex interactions between immune regulatory cells. Indeed, in recent years, there have been considerable advances in the understanding of immunoregulatory components.

The emerging role of several distinct populations of Treg cells in addition to CD4+CD25+ Treg, including iNKT, CD8+ inhibitory T cells, NK cells and the discovery of important new players such as IL-17 secreting (Th17) CD4+ T cells or gamma/delta T cells increase the complexity (227, 232, 236). Estrogen treatment in mice induces a novel population of suppressive regulatory cells, likely corresponding to a NKT subset (237, 238). All these cells may be new appealing targets integrating the various influences of sex steroid hormones on the immune system. Interestingly, stimulated female T lymphocytes secreted more IL-17 than male T lymphocytes indicating that female T lymphocytes exhibit not only more robust Th1 but also Th17 responses than their male counterparts (233). Deficiency in

endogenous IL-12 production from antigen presenting cells within lymph nodes of male SJL mice might also account for the gender differences in the induction of EAE (239, 240). Interestingly, gamma/delta T cells provide an important signal for the production of IL-12 by macrophages via cell-cell interactions (236). Further studies are needed to uncover whether this cell population could be held accountable for the sex differences in immune function.

NK cells are another lymphocytic population recognized to have a crucial role in shaping innate as well as adaptative immunity. Besides their cytotoxic activity, and cytokine production, the interaction of NK cells with dendritic cells is important for generating fully mature DCs able to induce a strong Th1 response (241). The sex dimorphism in immune responses may be partly explained by the increased frequency of a NK cell subset in SJL males compared with females as NK cells play a role in maintaining the male Th2 environment via an alteration in the antigen presenting cell function of peritoneal macrophages (199). Long-term estrogen treatment in mice or pregnancy are well known to suppress lymphopoiesis in the bone marrow leading to the decreased production of bone marrow derived cells including NK cells and natural killing activity (198). In vitro data have led to discrepancies in the effects of sex steroids in cytolytic activity and proliferation of NK cells which may be due to the presence of different NK subsets. Nethertheless, murine splenic NK cells express both ERalpha and ERbeta and experimental data clearly indicate that estrogen, from early pregnancy levels, can directly act on these cells to suppress NK cell cytotoxic activity mostly through ERbeta (205). Progesterone favors Th2, inhibits Th1 development and suppresses NK cell cytolytic activity that may be under the control of the Progesterone-Induced Blocking Factor secreted by gamma/delta or CD8+ T cells (215, 242). Experimental data in healthy men indicate that testosterone and/or its metabolites, including estradiol, may suppress NK cell proliferation (200). Further studies in both rodent and human are needed to better understand the influence of sex steroids on NK cells in DC interaction and maturation.

Taken together, the effects of sex steroids (at least at physiological high doses) on T cell cytokine profiles in vitro and T regulatory/suppressive functions partly explain their beneficial effects on MS/EAE development. It should be kept in mind that the absence of gender differences in some EAE models and mouse strains may be due to the use of pertussis toxin, a bacterial toxin, often needed as an adjuvant to promote disease development. While acute enhancement of vascular permeability to pathogenic T cells and of Th responses have been implicated in this effect, several other long lasting actions critical to the development of clinical symptoms, occurs following acute pertussis toxin injection. Indeed, pertussis toxin stimulates the maturation of the antigen presenting cells, i.e. macrophages or DCs, via Toll-like receptor 4 signaling (243–245). It leads to defects in T cell anergy to myelin peptides via depletion of splenic CD4+ Foxp3+ Tregs and concomitant expansion of T effector cells (Th1, Th2 and Th17) (243, 244, 246–250). While these effects have clearly revealed the potential role of microbial components in dysregulating the homeostasis of the immune system, their use in rodent models, when assessing the influence of sex steroids on disease development, may affect EAE sexual dimorphism by overriding important genetic checkpoints, notably the ones controlling Treg function in the pathogenesis of the disease (251).

Besides, chronic administration of high levels of estrogen leads to bone marrow aplasia and thymus involution, organs also involved in autoreactive cell deletion (187, 252, 253). Indeed, high estrogen administration reduces the pool of early thymic precursors in the bone marrow and thymus as well as the proliferation of CD4/CD8 double positive thymocytes. It can also reactivate an extrathymic pathway of T cell differentiation in the liver and spleen, where autoreactive cells might develop in the absence of negative selection, potentially increasing the risk of autoimmunity on a long term (187, 254–256).

B cells

While chronic administration of high levels of estrogen or pregnancy suppresses B lymphopoiesis, ovariectomy or orchidectomy induces B lymphopoiesis in the mouse bone marrow (252, 257, 258). B cells express intracellular but not membrane steroid receptors (ER and AR) with higher levels of ERbeta compared to ERalpha (210, 259). Female sex hormones induce B cell activation by increasing the secretion of IL-6 and IL-10 which induce B cell proliferation, and immunoglobulin production by promoting B cell maturation (260–262). High levels of estradiol (1–100 nM) potentiates the antigen-specific primary antibody response from human peripheral blood mononuclear cells by inhibiting CD8+ T cell mediated suppression of B cells (263). Similarly, estrogen had a dosedependent stimulatory effect (30–3000 pM) while progesterone had a dose-dependent inhibitory effect (30–3000 nM) on the frequency of immunoglobin-secreting cells in peripheral blood mononuclear cell cultures from female rhesus macaques, and these changes required the presence of CD8+ cells (264). The effects of progesterone on B cells are thought to be mediated in part by the immunoregulatory action of Progesterone-Induced Blocking Factor secreted by gamma/delta or CD8+ T cells (215).

Estrogen decreases the early hematopoietic progenitor pool and induces a shift toward a mature B cell subpopulation mainly through ERalpha (265, 266). Non hematopoietic cells - stromal cells in bone marrow - expressing ERbeta and AR are also important for sex steroid-mediated suppression of B lymphopoiesis (217, 267). Splenic B cells from normal mice undergo apoptosis unless rescued by stimulation. Estrogen induces polyclonal B cell activation and B cell resistance to apoptosis via upregulating Bcl-2 expression (265, 268–270). As a consequence, exogenous estrogen alters tolerance induction of naive immature B cells and enhances the survival of autoreactive B cells normally deleted or anergized (268). Thus, estrogen may override immune tolerance to self-antigens, exacerbating autoimmune disease in which autoreactive B cells are involved (187 for review). The participation of B lymphocytes in MS pathology has been

proposed after observing increased intrathecal IgG production, the presence of B lymphocytes and of autoantibodies directed against myelin in active demyelinating lesions (271). Though autoreactive B cells do not appear to be critical for EAE development, they contribute to EAE/MS disease severity by producing pathogenic CNS specific-autoantibodies believed to exacerbate the disease (253, 272, 273). Moreover, they may play an important role in MS disease variants such as Devic disease or in cortical pathology in secondary progressive MS (274, 275). On the other hand, recent studies have also highlighted the tolerogenic role of B cells acting as antigen presenting cells: B cells interact with and expand CD4+ CD25+ Treg cells through a B7-dependent mechanism that causes the cells to get mobilized and to migrate into the CNS, leading to EAE disease resolution by producing IL-10 (276–278). Further studies are required to determine the effects of sex hormones on B cell function and elucidate the overall actions of sex steroids on B cells during MS.

Mast cells

Mast cells, classically associated with allergy, may also contribute to the pathogenesis of the disease in both human and animal models (4, 279–283). Histamine and platelet activating factor secreted by mast cells facilitate the CNS entry of autoreactive T cells by increasing blood–brain barrier permeability (284). Apart important immunoregulatory cytokines that can be released by mast cells, histamine itself can polarize the immune response toward Th1 through histamine receptors on lymphocytes or CNS cells (285–287). Mast cells may also directly participate in the destruction of myelin by secreting proteases (4, 288). ER and PR expression in mast cells has been shown by immunocytochemistry and RT-PCR (289, 290). Only, few studies have examined the effects of physiological doses of sex steroid hormones on mammalian mast cells in vitro. Estradiol (10–100 nM) inhibited TNF-α and IL-6 release from a human mast cell line (291). In contrast, only at concentrations between 10 pM and 1 nM, estradiol increased the release of allergic mediators, leukotriene C4 and beta-hexosaminidase, via a non-genomic ERalpha mediated pathway (290). Progesterone (100 nM-1 μM) inhibits histamine secretion from activated rat mast cells and reduces the CXCL12-mediated migration of mast cells (292, 293). Thus, mast cell activation and migration are significantly affected by sex hormones. In view of the relatively recent implication of the 'allergic' arm in autoimmune demyelinating disorders, further studies are required to better delineate the potential effects of sex steroid hormones on mast cell mediators in regards to immune functions and myelin degradation.

Antigen presenting cells

The sexual dimorphism of the immune response is also mediated indirectly through antigen presenting cells (APC), such as macrophages and dendritic cells (DCs).

Infiltrating macrophages and the resident CNS macrophages, the microglia, which may also differentiate into DCs, play a pivotal role in the production of toxic inflammatory mediators and destructive mechanisms leading to demyelination and axonal damage in EAE/MS (294, 295). Macrophages derived from males and females express equivalent levels of mRNA encoding proinflammatory cytokines such as IL-1beta, IL-18, TNFalpha, and IL-12 (296). However, they exhibit gender dimorphism in cytokine production after T cell activation, with cells isolated from female or castrated male (SJL) mice secreting preferentially IL-12 in contrast to male APC secreting preferentially IL-10 (240). Low levels (30 pM) of estradiol or progesterone increase - while higher levels (>0.3 nM) reduce - TNFalpha release from peritoneal macrophages. In contrast, testosterone had no effect (297). ERalpha but not ERbeta in macrophages plays a predominant role in mediating the inhibition of matrix metalloproteinase-9 and the production of cytokines such as IL-6 and TNFalpha production by estrogen. This effect involves alterations in the NFkB and/or MAPK signaling pathways (298–301). Moreover, ERalpha-deficient splenic macrophages, but not ERalpha-deficient CD11c+ splenic dendritic cells, enhance the T cell proliferative response and IFN-gamma production compared to wild-type APC (302).

The AR is also expressed on peripheral macrophages with a 4–8 fold higher expression in male than female rodent or human (151, 303 , 304). Indirect, membrane sex steroid signaling has been reported through intracellular calcium regulation. In murine RAW 264.7 macrophage cell line, estradiol and testosterone induce a rapid rise in the intracellular free calcium concentration via membrane ER and AR respectively, down-regulate the serum-induced c-fos promoter and ERK1/2 activation, but up-regulate the lipopolysaccharide-stimulated activation of c-fos promoter, p38, and nitric oxide (NO) production indicating different effects on macrophage upon activation inducer (305, 306). Whether sex steroid modulation of NO production by CNS infiltrating macrophages is beneficial or harmful for EAE/MS remains a complex issue detailed elsewhere (307–309).

Several recent reports also suggest that estrogen and progesterone regulate disease progression through modulation of DCs, critical mediators of adaptive immunity, tolerance and autoimmunity. They are the primary APC directing T-cell function and activating autoreactive CD4+ T cells. However, DCs exposed to antigens in the absence of full-maturation stimuli down-regulate immunity and induce Treg cells, contributing to T cell tolerance. DCs are present within secondary lymphoid tissues as well as in the CNS, thus potentially sampling CNS antigens. DCs found within MS lesions have been shown to be functionally abnormal. The reader is directed to the recent review by Manuel et al that provides an insightful perspective on DC role in controlling tolerance and autoimmunity (310).

Cultured mouse splenic DCs express high levels of intracellular ERalpha (311). Estradiol (~2–10 nM) reduces TNFalpha, INFgamma and IL-12 production by mature CD11c+ DCs and prevents them from presenting antigen to myelin basic protein-specific T cells, as

assessed by their reduced ability to induced T cell proliferation in mouse mixed lymphocyte reaction assays (311, 312). Culturing splenic DCs with estradiol in addition to GM-CSF and IL-4, cytokines classically used for their expansion and maturation in vitro, did not affect their expression of the surface markers CD11b, CD11c, CD25, CD80, CD86, and DEC205 (311). This suggests that estrogen has no effect on the maturation of DCs. However, coculture of encephalitogenic T cell clones with E2-pretreated DCs resulted in a decreased percentage of TNFalpha or IFNgamma producing CD4+ T cells and an increased percentage of IL-4 and IL-10 producing CD4+ T cells (311). Interestingly, estradiol at ~2 nM, levels corresponding to early pregnancy, up-regulated rat DC expression of indoleamine 2,3-dioxygenase, which has been associated with tolerogenic properties (313). Moreover, splenic DCs obtained from EAE Lewis rats and exposed in vitro to late pregnancy levels of estradiol protected Lewis rats from acute EAE as indicated by the decreased severity of clinical symptoms (313). This effect was associated with a reduction in circulating CD4+ cells concomitant with a slight increase in circulating CD4+IL10+ T cells and CD8+CD28− suppressor T cells. If the beneficial effects of estrogen-treated DCs are further confirmed, a new therapeutic avenue might be opened. In vitro, encephalitogen-activated blood mononuclear cells recovered from estrogen (vs. control) exposed- DC treated rats showed an increased secretion of IL-10 and IFNgamma and decreased proliferation (313).

These studies contrast with the effects of low estrogen levels (0.1 nM), which have been shown to promote Granulocyte Macrophage Colony Stimulating Factor (GM-CSF)-mediated differentiation of DCs from bone marrow progenitors issued from either male or female mice. These cells also kept their antigen presentation capability and their ability to stimulate the proliferation of naïve CD4+ cells in vitro (314). As emphasized by Paharkova-Vatchkova et al, a stimulatory effect has been (dis)missed in previous studies likely due to improper culture conditions such as use of serum not depleted from steroids (314). More recently, estradiol through ERalpha (but not ERbeta) has been shown to be critical for the normal DC development from BM precursors (315).

Progesterone, at concentrations similar to that seen during the ovarian cycle or pregnancy, inhibits the ability of bone marrow-derived mature DCs obtained from female rodents to express the cell-surface co-stimulatory CD80 and MHC class II molecules, to secrete pro-inflammatory cytokines (TNFalpha, IL-1beta) and to stimulate T lymphocyte activation, while it affects slightly antigen uptake ability by immature DCs only at pregnancy levels (316). There were no significant changes in surface marker expression or T cell stimulatory capacity of DCs which were derived from blood immature DCs and matured in vitro under the influence of high physiological levels of progesterone and/or estradiol (317). Interestingly, progesterone or estradiol also increased IL-10 and decreased IL-18 production from rodent and human DCs (316, 317).

Thus, estrogen and progesterone appear to have multiple effects on DCs depending on dose and maybe DC subsets, but likely favor their tolerogenic properties and the down-regulation of Th1 activation when sufficient hormone levels are achieved.

Endothelial cells

Increasing evidence suggests that sex steroids may act by regulating the permeability of endothelial cells which compose the blood brain barrier (318). In particular, several studies have now demonstrated that endothelial cells are indeed a target for estrogen action, mainly through ERalpha via genomic as well as non genomic pathways (318, 319). Estradiol has been shown to stimulate prostacyclin production accounting for its atheroprotective action and to increase endothelial cell permeability to albumin, water, insulin and sugars (319, 320 for review). Acute treatment with pregnancy levels of estradiol has been shown to increase the expression of endothelial adhesion molecules, favouring leukocyte binding to endothelial cells, a first step in leukocyte entry into the parenchyma (321). In contrast, high and prolonged estrogen treatments as well as progesterone decrease the gene expression of cytokine-induced adhesion molecules in cultured endothelial cells or isolated cerebral vessels (322, 323, and 189, 320 for reviews). Recent data suggest that the inhibitory action of estradiol (and testosterone after aromatization) on TNFalpha-induced vascular cell adhesion molecule expression depends on ERbeta signaling (324, 325). Most of these studies have been assessed in human umbilical vein endothelial cells or human coronary artery cells, and only in recent years the properties of brain endothelial cells have been examined. Because cerebral endothelial cells interact with astrocytic endfeets, an important feature for barrier tightening of the cerebral microvasculature, further studies are needed to address the direct effects of sex steroids on this specific endothelium. Estradiol has been reported to inhibit the migration of inflammatory cells in a rat carotid (artery) injury model (326). However, an in vitro study has indicated that estrogen acts synergistically with myelin basic protein to cause mast cell infiltration into the brain parenchyma (288). Moreover, estrogen stimulates the expression of brain derived neurotrophic factor (BDNF) which can induce the release of inflammatory mediators by mast cells (327). This would rather be harmful in the context of multiple sclerosis. On the other hand, estrogen exhibits several protective effects via cerebral endothelial cells. Indeed, estradiol reduces edema formation and ischemia- and vascular endothelial growth factor-induced blood-brain barrier disruption, in contrast to testosterone (328–330). Various mechanisms have been suggested to account for these protective effects, including decreased expression/activity of electrolytes tranporters, adhesion molecules or matrix metalloproteases, increased expression of occludin, a tight junction protein and modulation of mitochondrial functions in cerebral blood vessels (165, 318, 329–331). These actions may partly account for the decreased infiltration of T cells or macrophages and DCs in secondary lymphoid tissue as well as in CNS from estrogen pretreated mice (129, 130, 142, 225, 311).

Taken together, the beneficial effects of estrogens, potentially preventing blood brain barrier leakiness during neuroinflammation, are likely to participate in the therapeutical estrogenic action during EAE/MS, though discrete proof of a direct action on brain endothelial cells is warranted.

Glial cells

Astrocytes and microglia

The classical nuclear receptors for sex steroid hormones are expressed in the CNS, including astroglia, though at much lower levels than in peripheral organs (332). Recent studies started to dissect the potential anti-inflammatory mechanisms of estrogens on glia. ERalpha and ERbeta are found in the nucleus and cytoplasm of cultured brain macrophages/microglia and astrocytes as well as reactive astrocytes and microglia in vivo (333–335). Membrane ERs have been also demonstrated on cultured astrocytes and microglia (336). In a microglial cell line, estradiol, as low as 1 nM, increases IL-10 and reduces TNFalpha and IFNgamma release from resting as well as activated cells (337). Recent evidence suggests that INFgamma can be produced by microglia in neuropathological conditions (338). These findings may be relevant to estrogen mediated dampening of neuroinflammation. In primary rodent astrocyte cultures, estradiol (1–10 nM) stimulates the expression and release of the neurotrophic and immunoregulatory factors such as TGFbeta from astrocytes via an ER-dependent mechanism involving the phosphoinositide 3-kinase/Akt signaling pathway (339). At higher concentrations (10–100 nM), estrogen increases the expression of glutamate transporters with functional consequences on glutamate uptake (340). Estradiol has also been shown to downregulate reactive gliosis in vitro and in vivo (341, 342). Estrogens suppress proinflammatory cytokines and NO release from activated microglia (343–346). In primary cultures of rat microglia, estrogen inhibits inducible NO synthase but also blocks the production of several other inflammatory signals, such as matrix metalloprotease-9 and prostaglandin-E_2 (347). The critical action of estrogen on microglia/macrophages can be explained in part by the ability of estradiol via ERalpha to prevent the translocation of NFkB subunits, blunting the trancriptional activity of the NFkB system, an important mediator of inflammatory cytokine production (300). While ERalpha seems to mediate most of the neuroprotective action via membrane and genomic mechanisms in astroglia (345), ERbeta may contribute as well (141, 348, 349). These examples indicate that sex steroids potentially prevent the amplification of inflammation in the CNS and contribute to neuroprotection by targeting several glial molecules.

Testosterone also down regulates reactive astrogliosis. This effect is largely due to its conversion into estradiol (341, 342). Androgen receptor signaling may act however on a subset of astrocytes and activated microglia in a region specific manner (342, 350–352). This effect correlates with the preferential expression of ER in forebrain astrocytes while activated microglia mainly expressed AR (335). These actions are in line with the neuroprotective effects of this steroid in some CNS disease models. However, dual “edge and sword” effects have been also ascribed to testosterone or its metabolites. For example, the classical intracellular/nuclear androgen receptor, once activated, promotes ERK and Akt phosphorylation, key effectors of neuroprotection-associated MAPK and phosphoinositide 3-kinase signaling pathways. On the other hand, the existence of a glial plasma membrane androgen receptor which suppresses ERK and Akt phosphorylation and promotes astrocytic cell death may explain why testosterone has brain damaging effects in some rodent models of neurotoxicity such as stroke (185, 186).

The expression of progesterone receptors PR-A/PR-B is rather low in the central nervous system and mostly confined to discrete neuronal populations inside the hypothalamus, hippocampus, brainstem and pons (353–356). Little is known regarding the astroglial expression of progesterone receptors or newly identified interacting binding proteins in the central nervous system. However, in the rodent spinal cord, moderate PR expression was found in both neurons and astroglial cells (182, 357). Moreover, an estrogen-induced expression of PR in forebrain astrocytes has been shown in culture (358), though this estrogenic regulation is not a general rule in the central nervous system (357, 359). Nethertheless, in vitro studies have shown that astrocytes and microglia are targets of progesterone, since it exerts some anti-inflammatory effects on these cell populations (e.g. regulation of inducible nitric oxide synthase expression) (182, 360, 361). Among the complex and pleiotropic actions of progesterone or its metabolites, it is worth noting that in vivo progesterone as well as allopregnanolone is able to reduce astrogliosis in animal models (341, 362, 363). Strikingly, estradiol is able to induce progesterone synthesis by astrocytes in culture (364), suggesting that estradiol may exert paracrine effects via progesterone.

Oligodendrocytes

Regarding the oligodendrocyte lineage, both ERalpha and ERbeta are expressed by oligoprogenitors and oligodendrocytes in vitro. ERbeta is mainly localized to the cytoplasm of oligodendrocytes and to the membranes of oligodendrocytes in vitro and to myelin (47, 365 , 366). Cultured oligodendrocytes express nuclear ER and long-term treatment with estradiol stimulates cell growth, process extension, and myelin basic protein expression (358). As pointed out before, the lifespan and turnover of rodent oligodendrocytes is shorter in females than in males (42, 47). Estradiol, but not progesterone or testosterone, was found to delay cell cycle exit of oligodendrocyte progenitor cells (47). Estradiol also enhanced myelin sheet formation in accordance with an early report, showing in vivo estradiol stimulatory effects on myelination (47, 367). Interestingly, estradiol, only at concentrations found during late pregnancy, protects oligodendrocytes in vitro from cell death induced by a cytotoxic agent (365). In contrast, only a subset of oligodendrocytes has been shown to express AR in the

primate or rodent central nervous system (350, 351). Testosterone, in the presence of aromatase inhibitor, amplified excitotoxic damage of oligodendrocytes in vitro (368). Progesterone increases oligodendroglial cell maturation and promotes remyelination (47, 182). Taken together, these data support a beneficial effect of estrogen and progesterone on oligodendrocyte maturation and survival in contrast to androgen signaling, in vitro.

Neuronal cells

Among the different neural cell types in the healthy nervous system, neurons express the highest levels of sex steroid receptors in several brain and spinal cord regions (333, 351, 369). In addition to the well known high expression of estrogen or androgen receptors in specific hypothalamic nuclei and brain areas related to reproduction, various degrees of ERalpha or beta and AR expression are observed among other neuronal populations including those in cortex, hippocampus or basal ganglia, with some subsets of motoneurons devoided of ER but showing substantial AR expression (333, 350, 369–376). Transcripts for GPR30, the G protein-coupled estrogen receptor 1, were also detected in several areas of the human and rat CNS (377, 378). This supports the idea that it may be an important receptor subtype through which estrogen exerts its effect. The distribution of GPR30 immunoreactivity in the CNS has been investigated only recently in rats and was associated with plasma membranes of neurons but also localized throughout the cytoplasm of some neuronal populations, notably in the Golgi apparatus (379–381). Despite the increasing complexity in the nature and localization of sex steroid hormone receptors and signaling, several lines of evidence support a direct neuroprotective effect of sex steroids, in particular estrogens via either non-genomic or genomic pathways. However, there are also reports indicating that estradiol exposure can be deleterious to some neuronal populations (333, 382, 383). Progesterone via PR signaling, sigma1 receptor binding modulating glutamate signaling, or its metabolite potentiating GABA signaling, is being an attractive molecule to dampen various CNS insults (182 for review, 384).

Neuronal damage is an important issue in multiple sclerosis and correlates best with persisting disability. Axonal and dendritic damage is detectable from early clinical stages, and is associated with inflammation and glutamate toxicity rather than demyelination per se. The expression of several neuronal genes such as ion pumps, synaptic proteins or mitochondrial proteins is indeed dramatically affected in the CNS during EAE or MS, in some cases, at very early stages of the diseases (385–388). Thus, several lines of evidence suggest that early neuroprotection should be implemented in MS patients in addition to current immunotherapies to prevent irreversible axonal loss and this is an active field of investigations. For the direct neuroprotective effects of estrogens, key potential targets are proteins involved in cell survival, axonal sprouting, regenerative responses and enhanced synaptic transmission. It is interesting to note that estradiol (10 nM) upregulates the expression level of synaptic proteins such as synaptophysin, syntaxin and synaptotagmin in neuronal cultures, likely through the MAP kinase pathway (389). In vitro, nanomolar concentrations of estrogen protect cultured neurons from various insults such as oxidative stress or glutamate toxicity (177, 390, 391). Interestingly, this effect is associated with a decreased expression of ionotropic glutamate receptor subunits (177). Estrogen and progesterone have also been shown to upregulate the expression of antiapoptotic proteins such as bcl-2 and of neurotrophic factors such as BDNF in rodent neural cultures via ERalpha and ERbeta (289, 327, 392 and 382 for review). Accordingly, ERalpha and ERbeta agonists or progesterone reduce neuronal damage induced by ischemia in rodents (393–397). There has been some evidence for a neuroprotective action of testosterone (398). However, its aromatization into estradiol may account for most of its action as emphasized before. Strikingly, testosterone at nanomolar concentrations, in presence of an aromatase blocker, amplifies NMDA-induced neurotoxicity in mixed mouse cortical cultures (399). This raises the possibility of harmful direct androgen signaling on neural cells. Taken together, these data suggest that natural sex steroids, by normalizing the expression of some key synaptic and mitochondrial proteins and the protective neuronal mechanisms, may provide effective direct neuroprotection in MS therapies.

SAFETY CONSIDERATIONS OF SEX STEROID TREATMENTS

Estrogens

Hormone replacement therapies (HRT) based on estrogens (estradiol, synthetic analogs, or CEE, a mixture of conjugated equine estrogens) and progestins are a complicated clinical issue that raises questions on risks vs. benefits, since the Heart and Estrogen/progestin Replacement Study (HERS) and Women Health Initiative (WHI) study found that HRT, despite having benefits on menopausal symptoms, increased the incidence of cardiovascular diseases rather than decreasing, as anticipated (400, 401). Several concerns have also been raised by the Million Women Study (MWS) about increased risks of ovarian and breast cancer (402). Increased risk of meningioma but not glioma and stroke as well as deficits in cognitive functions have also been attributed to HRT (182, 403–405). Still, it is difficult to extrapolate these results to the various HRT regimens that are used and that differ in their doses, compositions and administration routes (404, 406). HRT regimens are quite different between North American and some European countries, where transdermal synthetic estrogens or oral micronized estradiol are preferred over oral conjugated equine estrogens. Notably, the anti-inflammatory activity differs substantially between conjugated equine estrogens and estradiol (407). The age of the patients enrolled in previous HRT studies (mid 60s) is another limit to actually extent the conclusions to younger populations. Clearly shown in rodents, a prolonged period of hypoestrogenicity disrupts the memory improvement and the neuroprotective and anti-inflammatory actions mediated by estradiol (408, 409). In fact, women initiating HRT closer to menopause likely respond better to hormone replacement compared to women more distant from menopause. Indeed, the WHI's second arm and WISDOM studies indicate that the excess cardiovascular risk is only confined to older women, which is consistent with previous observational studies in which women started HRT early (410–412). Similarly, the Cache

county study has evidenced different outcomes for the risk in Alzheimer disease in women initiating HRT before or after age 64 (413). The phase IV Kronos Early Estrogen Prevention Study (KEEPS) and phase II/III Early vs. Late Intervention Trial with Estradiol (ELITE) will address these debated issues by examining the benefit of estrogen replacement (oral CEE versus transdermal estradiol, both in combination with progesterone for KEEPS; oral estradiol with vaginal progesterone gel for ELITE) on cardiovascular disease and cognition in recently menopausal women (414). SYMPTOM, another trial in Finland, will compare oral and transdermal HT on vascular and cardiac function in recently menopausal women. No doubt, the MS field will benefit from such studies even if the risk-benefit ratio of estrogenic treatments for neuroinflammatory disorders such as MS is much different than for preventive use in healthy peri- and post-menopausal women.

Another issue in the HRT field is the safety of estriol versus estradiol or equine estrogens. Estriol, a metabolite of estradiol and a major estrogen produced by the placenta cannot be converted to estradiol. Despite claims that it is safer than other forms of estrogen, overall evidence suggests that estriol, often mislabeled as a weak estrogen, likely presents the same risks as other estrogens when taken alone at high doses. It is thus uncertain whether estriol alone is safer than estradiol, in particular in postmenopausal women, whose natural estrogen levels have dropped. Indeed, estriol binds to estrogen receptors though with lower affinity than estradiol and is considered a partial agonist (415). Chronic administration of estriol produces full estrogenic responses in animal models as well as in vitro in the absence of estradiol (416–418). In contrast, a 10-fold molar excess of estriol over estradiol induces anti-estrogenic activity (418). Indeed, when estriol is given in doses equivalent to estradiol, but administered more frequently to compensate for its rapid excretion, estriol also increases the relative risk of endometrial cancer and endometrial atypical hyperplasia in postmenopausal women (419). Further clinical studies based on monitoring blood estriol/estradiol ratio are warranted to assess the safety of estriol and effective dosages, depending of the patient's ovarian cycle status.

Progestagens

It is yet unclear which progestagen (progesterone, progestins) will lead to the best outcome in recent menopausal women. Progestins, drugs with progesterone-like actions on the uterus, are commonly used with estrogens in HRT for menopausal women to prevent hyperplasia of the endometrium and reduce the risk of endometrial cancer associated with estrogen supplementation. The rationale is that progesterone exerts inhibitory effects on estrogen signaling by decreasing the number of estrogen receptors in reproductive organs and increasing its conversion to inactive metabolites. However, progestins commonly used in humans in North America and European countries, including 17alpha-progesterone derivatives such as MPA; 19-nortestosterone derivatives; 19-norprogesterone derivatives, have differential anti-inflammatory or neuroprotective actions compared to the natural progesterone (182 for review). Because the progesterone receptor agonist MPA also binds to the glucocorticoid receptor, it can exhibit additional immunoregulatory properties such as inhibition of interleukin 1, 2 and 6 production (420). In vivo and in vitro studies suggest differential actions of MPA and progesterone in endothelial cells, vascular smooth muscle cells, and neurons. Indeed, in vascular endothelial cells, progesterone but not MPA, reduced vascular cell adhesion molecule-1 expression (323). Progesterone plus estradiol, but not MPA plus estradiol, protect against coronary artery vasospasm in ovariectomized rhesus monkeys (421). Moreover, recent findings in rodents indicate that certain progestin formulations, in contrast to progesterone, affects the vulnerability of the central nervous system to degenerative insults. Indeed, Nielsen et al found that estradiol and progesterone exert neuroprotection against glutamate neurotoxicity, while MPA antagonizes the neuroprotective effect of estradiol and exacerbated neuron death induced by glutamate excitotoxicity (422–424). These few examples suggest that the disparities between progestins and progesterone, may be due to differential, agonist-specific changes in progesterone receptor conformation and transcriptional activities, progesterone receptor isoform selectivity, or membrane vs. nuclear progesterone receptor signaling (182). Which progestagen formulation is best for coadministration with estrogens to treat neuroinflammatory diseases remains at present unresolved. From EAE experimental data, one can even ask whether progestagens, despite potential beneficial effects on immune system, myelination and neuroprotection during ischemia, will not be harmful for the neuroprotective and anti-inflammatory actions of estrogens in the context of multiple sclerosis. Nethertheless, further research on progesterone receptor agonists with clear neuroprotective effects in the context of neuroinflammation is warranted.

CONCLUSION AND PERSPECTIVES

The determination of the cell types that are targets of sex steroids and the elucidation of the underlying mechanisms are of paramount importance to unravel the causes of gender differences in multiple sclerosis and to design the most appropriate therapeutic approaches. Sex hormones have bimodal effects on the immune system and the anti- vs. proinflammatory effects of estrogen in particular depend of the dose and the time point of estrogen administration in relation to the state of the inflammatory disease. Sex steroids regulate the differentiation, maturation and function of many cell types directly or indirectly by autocrine/paracrine mechanisms. Thus, whether sex steroids exert stimulatory or inhibitory effects on the overall neuroinflammatory process is a function of cell specificity, determined by expression of receptor subtypes and splice variants, nuclear vs. membrane/intracytoplasmic receptor signaling, as well as the interactions between cells and the local milieu. Experimental, clinical and MRI evidence have indicated a link between sex steroid hormones including estradiol, testosterone and progesterone, and multiple sclerosis. It has been suggested that sex steroid supplementation can be beneficial via the immunoregulatory, anti-inflammatory and neuroprotective properties. However, it should be kept in mind that not all estrogens and progestins are equal in action. Clearly, further basic science information is crucial to our understanding of the immune and neural

implications of clinically used sex steroid receptor modulating drugs. The outcomes of the different ongoing MS clinical trials may help to find the best use of sex steroids in combination with current therapeutic drugs. Newly developed synthetic selective estrogen response modulators (SERMs) may also provide the protective effects without harmful effects. Indeed, several new steroid hormone analogs and SERMs are now under consideration such as selective ERalpha vs. ERbeta agonists, ER modulators without the undesired uterotropic activity (135, 425–427). Such studies will certainly be of pivotal importance to the design of new sex steroid-based therapeutic approaches for MS.

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Abbreviations

Ag: antigen
APC: antigen presenting cell
AR: androgen receptor
CNS: central nervous system
DC: dendritic cell
DHT: 5alpha-dihydrotestosterone, EAE, experimental autoimmune encephalomyelitis
ER: estrogen receptor
ERE: estrogen response elements
HPA: hypothalamic-pituitary-adrenal axis
HRT: hormone replacement therapy
MAPK: mitogen activated protein kinase
MPA: medroxyprogesterone acetate
MRI: magnetic resonance imaging
MS: multiple sclerosis
NFkB: nuclear factor kappa B
PBMCs: peripheral blood mononuclear cells
PR: progesterone receptor
Th1: T helper cell type 1
Th2: T helper cell type 2
TMEV: Theiler's murine encephalomyelitis virus
Treg: regulatory T cell

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Table 1

Gender differences in multiple sclerosis rodent models (Lewis or Wistar rats and various mouse strains).

Strain (haplotype)	model	EAE onset	EAE incidence	EAE severity	Notes	Ref.
Lewis rat	active EAE	n.a.	n.a.	n.a.	RR in F, acute monophasic in M	13
Lewis rat	active EAE (+ cyclosporin)	n.a.	n.a..	n.a.	RR in M, acute monophasic in F	14
Wistar rat	Active EAE	F=M	F=M	F=M	GDX in males ↑ disease duration while delaying onset	428
SJL (H-2^s)	active EAE	n.d.	F=M	F=M		15
C57BL/6 (H-2^b)	active EAE	F=M	F=M	F=M	GDX in males → disease severity	20, 150, 429
C57BL/6	active EAE	earlier in F	F=M	F=M	onset varies upon estrus cycle at time of immunization	79
NOD (H-2^{g7})	active EAE	F=M	F=M	F=M		20
	active EAE	F=M	F=M	F=M		20
SV.129 (H-2^b)	active or passive EAE	F=M	F=M	F=M	only males sensitive to PPAR deletion, earlier onset when PPAR$^{-/-}$ CD4+ cells are used for transfer	233
SJL	active EAE	F=M	F=M	F>M	RR in F and GDX M vs. acute monophasic in intact M	16, 17, 21
SJL	active EAE	earlier in F	F>M	F>M	GDX in males ↑ disease severity	20
SJL	active EAE		F>M		GDX in males (but not in F) ↑ disease severity	150
SJL	passive EAE	earlier in F		F>M		18
SJL	passive EAE	earlier in F	F≫M	F>M	Male splenocytes transfer disease less effectively than their F counterpart	17, 21
ASW (H-2^s)	active EAE	F=M	F=M	F>M		20
NZW (H-2^d)	active EAE	F=M	F>M	M>F		20
F2 B10.S × SJL crosses (H-2^s)	active EAE	F=M	F=M	F>M	F develop more often the chronic form than M; GDX ↓ disease severity and duration in F while slightly ↑ severity in M	31
B10.PL(H-2^u)	active EAE	F=M	F=M	F=M, M>F	↑ mortality or severity during the early phase in M and GDX F compared to intact females	20
B10.S (H-2^s) Treg suppressed	active EAE		M>F	M>F	CD25 blocking antibody more effective in M than F	196
C57BL/6	TMEV-D		M≫F			23
SJL	TMEV-D			M>F	↑ antiviral antibody responses in F	58
SJL	TMEV-D			F>M (by histology)	F more susceptible to TMEV-induced CNS damage	26
C57BL/6	TMEV-D		M≫F		M unable to clear the virus from the CNS in contrast to F	24
C57BL/6	TMEV-D		M≫F		castration ↑ disease	25

Abbreviations: CNS, central nervous system; F, female; GDX, gonadectomy/gonadectomized; M, male; PPAR, Peroxisome proliferator-activated receptor alpha; RR, relapsing remitting form of EAE;; TMEV Theiler's murine encephalomyeltis virusinduced demyelination. ↑, increase; ↓, decrease; →, no variation; n.a., not addressed due to low number of rats or qualitative differences in EAE profiles.

Table 2

Range of human and mouse circulating sex steroid concentrations.

Species	Sex/status	E2 (pg/ml)	E2 (nM)	E3 (pg/ml)	E3 (nM)	Pg (ng/ml)	Pg (nM)	T (ng/ml)	T (nM)
human	follicular phase	20–150	0.1–0.5	10–20	0.03–0.07	0.1–1.5	<5	0.3–1	1–3
human	mid-cycle phase	100–500	0.4–2	10–20	0.03–0.07	~ 1.5*	~ 5*	0.3–1	1–3
human	luteal phase	50–300	0.2–1	10–20	0.03–0.07	2–24	8–80	0.3–1	1–3
human	6/7 months pregnant	7,500–12,500	30–50	4,000–6,000	15–20	60–90	200–300	0.3–1	1–3
human	9 months pregnant	12,500–25,000	50–100	6,000–24,000	20–80	100–200	300–600	0.3–1	1–3
human	man	10–75	0.03–0.25	50–90	0.2–0.3	0.1–0.3	0.3–1	3–10	10–30
mouse	Diestrus	20–50	0.1–0.2	~ 50	~ 0.2	1–7	3–20	<1	<3
mouse	Estrus	100–200	0.5–1	~ 50	~ 0.2	1–7	3–20	<1	<3
mouse	late pregnancy	5,000–10,000	20–40	1,500–3,000	5–10	50–100	200–300	1–2	3–6
mouse	male	8–25	0.03–0.1			1–2	3–6	3–10	10–30

Concentrations of sex steroid hormones measured in human ([156], [215], [430_434]) or rodent ([127], [152], [435], [436]) blood or plasma.

*, 17-hydroxy-progesterone. E2, estradiol; E3, estriol, Pg, progesterone; T, testosterone

Table 3

Effect of estrogen administration on mouse EAE development with respect to Th1/Th2 cytokine profiles and neurohistopathology.

Strain	Sex	EAE Model	Compound (levels)	EAE onset	EAE incidence	EAE severity	Immunologic parameters	Neuropathologic parameters	Ref.
B10.RIII (H-2^r)	F[1]	Active	E2 or E3 (pregnancy)	Delay	n.d		n.d.	n.d.	437
SJL	F	Passive	E3 (pregnancy)	Delay	↓	↓	↑ IL-10 secretion by splenocytes, ↑ IgG1 serum levels (Th2 bias)	↓ neuroinflammation	127
B10.PL, TCR Tg[2]	F[1]	Active	E2, E3 (various)	Delay	→	↓	↓ (trend) INFgamma production by splenocytes, ↓ IgG1 and IgG2a serum levels	n.d.	438
SJL, BP10.PL	M, F	Active	E2, E3 (various)	Delay	(↓)	↓	↑ (trend) IL-10 production, ↓ (trend) INFgamma production by splenocytes	↓ neuroinflammation, ↓ demyelination	142
C57BL/6	F	Active	E2 (pregnancy)	Delay	↓	↓/→ [3]	↓ % of TNFalpha+ T cells from CNS or spleen, → Th1/Th2 cytokine profile of lymph node cells	↓ CNS recruitment of inflammatory cells, ↓ % of TNFalpha+ macrophages/microglia	128, 129
C57BL/6	M	Active	E3 (pregnancy)	→	→	→	↓ TNFalpha and INFgamma and ↑ IL-5 secretion by splenocytes	n.d.	131
C57BL/6 (B6)	F	Active	E3	no EAE	no EAE	no EAE	↓ TNFalpha and INFgamma secretion by splenocytes	n.d.	131
C57BL/6	F	Active	E2 (pregnancy)	Delay	↓	↓	↓ TNFalpha and INFgamma secretion by splenocytes	↓ neuroinflammation	134
B6.129 ERalpha −/−	F	Active	E2 (pregnancy)	Delay[4]	→	→	↑ (trend) TNFalpha and INFgamma secretion by splenocytes	→ neuroinflammation	134
B6.129 ERbeta−/−	F	Active	E2 (pregnancy)	Delay	↓	↓	↓ TNFalpha and INFgamma secretion by splenocytes	↓ neuroinflammation	134
SJL	F	Active	EE or E2 (pregnancy)	Delay	↓	↓	↓ INFgamma secretion by splenocytes, ↓ IgG2a (but not IgG1) serum levels	↓ neuroinflammation, ↓ demyelination	130
C57BL/6	M, F (GDX)	Active	E2 (diestrus or pregnancy)	no EAE	no EAE	no EAE	→ TNFalpha or INFgamma secretion from CD4+ cells[5]	↓ neuroinflammation	136
B6 ERalpha$^{-/-}$	M, F (GDX)	Active	E2 (diestrus or pregnancy)	→	→	→	→[5]	→ neuroinflammation	136
B6.129	F	Passive	E2 (pregnancy)	no EAE	no EAE	no EAE	n.d.	↓ neuroinflammation, ↓ demyelination	137
B6.129 ERalpha$^{-/-}$	F	Passive	E2 (pregnancy)	→	→	→	n.d.	→ neuroinflammation, → demyelination	137
SJL	F (GDX)	Passive	E2 or ERalpha ligand (low)	Delay	↓	↓	↓ Th1 and Th2 cytokine profiles of splenocytes	n.d.	135
C57BL/6	F (GDX)	Active	E2 or ERalpha ligand (low)	no EAE	no EAE	no EAE	↓ TNFalpha and INFgamma secretion, ↑ IL-5 secretion by splenocytes	↓ neuroinflammation, ↓ demyelination, ↓ neuronal damage	132, 141
C57BL/6	F (GDX)	Active	ERbeta ligand	→	n.d.	→/↓ [6]	→ Th1/Th2 cytokine profiles of splenocytes	→ neuroinflammation, ↓ demyelination, ↓ neuronal damage	141

Abbreviations: EE, ethinyl-17alpha-estradiol; E2, estradiol; E3, estriol; F, females; GDX, gonadectomized; M, males; n.d., not determined or not applicable. ↑, increase; ↓, decrease; →, no significant variation.

1 Earlier onset in ovariectomized mice;

2 Transgenic mice bearing the functionally rearranged BV8S2 gene specific for MBP-Ac1-11 used as encephalitogen;

3 The estradiol treated mice showing clinical signs of disease reached the same level of severity as control mice;

4 Possibly due to the slight increase in estradiol plasma levels;

5 comparing cells isolated from spleens of vehicle and of low estradiol treated EAE ovariectomized mice;

6 Reduction after the acute initial phase.