[Frontiers in Bioscience 14, 4477-4515, January 1, 2009]

Gender and sex hormones in multiple sclerosis pathology and therapy

Arnaud Nicot[1,2]

[1]*INSERM UMR S 546, Paris, F-75013 France,* [2]*Universite Pierre et Marie Curie-Paris6, Paris, F-75013 France*

TABLE OF CONTENTS

1. 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.

2. 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 self-reactive 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.

3. INFLUENCE OF GENDER ON SUSCEPTIBILITY TO MS AND ITS ANIMAL MODELS

3.1. 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.

3.2. 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).

3.2. 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 Figure 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

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 x 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 slighlty ↑ 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-D: Theiler's murine encephalomyeltis virus-induced demyelination. ↑, increase; ↓, decrease; →, no variation; n.a., not addressed due to low number of rats or qualitative differences in EAE profiles.

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 histoneuropathological scores than males in accordance with EAE data (26).

3.3. 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

Reference	Strain (MHCII haplotype)	Model	Sex dimorphism			Notes
			Onset	Incidence	Severity (score or duration)	
Rats						
13	Lewis	Active EAE	not spefically addressed*			RR in females, acute monophasic in males
14	Lewis	Active EAE (+cyclosporin)	not spefically addressed*			RR in males, acute monophasic in females
428	Wistar	Active EAE				Castration in males increased disease duration while delaying onset
Mice						
No sex differences						
15	SJL	Active EAE	n.d.	F=M	F=M	
20, 150, 429**	C57BL/6 (H-2^b)	Active EAE	F=M	F=M	F=M	No increased disease severity in castrated males
79	C57BL/6	Active EAE	Earlier in F	F=M	F=M	Onset varies upon estrus cycle status at time of immunization
20	NOD (H-2^{g7})	Active EAE	F=M	F=M	F=M	
20	PL/J (H-2^u)	Active EAE	F=M	F=M	F=M	
233	SV.129 (H-2^b)	Active EAE	F=M		F=M	Only males sensitive to PPAR deletion
		Passive EAE	F=M		?	Earlier onset when PPAR deficient CD4+ cells are used
Female predilection						
16, 17, 21	SJL (H-2^s)	Active EAE				Relapsing form in castrated males vs. monophasic disease in intact males
20	SJL	Active EAE	Earlier in F	F>M	No relapse in males	Increased disease severity in castrated males vs. intact males
150	SJL	Active EAE		F>M		Increased disease severity in castrated males vs. intact males; no effect of ovariectomy
18	SJL	Passive EAE	Earlier in F		F>M	
17, 21	SJL	Passive EAE	Earlier in F	F>>M	F>M	Male spleen cell less effective in transfering disease than their female counterpart
20	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	
31	F2 crosses (H-2^s) (B10.S x SJL)	Active EAE	F=M	F=M	F>M	Females develop more often the chronic form than males. Decreased disease severity and duration in ovariectomized mice; slight increase in disease severity in orchiedctomized mice
Male predilection						
20	B10.PL (H-2^u)	Active EAE	F=M	F=M	F=M , M>F***	
196	BP10.S (H-2^s)	Treg "depleted" mice		M>F	M>F	CD25+ blocking antibody has more influence on EAE development in males than females
TMEV-D in mice						
23	C57BL/6 background			M>>F		
58	SJL				M>F	SJL females producing more antiviral antibody
26	SJL		nd	nd	F>M****	SJL unable to clear the virus from the CNS
24	C57BL/6 background			M>>F		Males unable to clear the virus from the CNS in contrast to females
25	C57BL/6 background			M>>F		Castration increases disease

Figure 1. Gender differences in multiple sclerosis rodent models. *, Low number of rats or qualitative differences in EAE profiles did not allow to address specically these issues; **, Two encephalitogens (MOG35-55 and MBP 18.5 kDa) were tested with similar results; ***, Increased mortality or severity during the early phase of EAE development in males and ovariectomized mice compared to intact females; ****, From histopathological scores. TMEV-D: Theiler's murine encephalomyeltis virus-induced demyelination

alpha gene polymorphism is associated with MS in Finnish and Japanese but not Italian populations (36-38).

3.4. 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 stressors such as opioid antagonist naloxone (an inducer of ACTH) 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 x 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 responseviness. 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.

4. HORMONAL FLUCTUATIONS AND DISEASE ACTIVITY

4.1. 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).

4.2. 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 (69) 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).

4.3. 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

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[1]	~ 5[1]	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. [1] 17-hydroxy-progesterone. E2, estradiol; E3, estriol, Pg, progesterone; T, testosterone.

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 immuno-regulatory 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).

4.4. The potential role of leptin

Another endocrine factor which may underly 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, is a significant determinant 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

		Estradiol		Estriol		Progesterone		Testosterone	
		pg/ml	nM	pg/ml	nM	ng/ml	nM	ng/ml	nM
Mouse	Diestrus	20-50	0.1- 0.2						
	Estrus	100-200	~ 0.5-1	~ 50	~ 0.2	~ 1-7	~ 3-20	< 1	< 3
	Late pregnancy	5,000-10,000	20-40	~ 1,500-3,000	~ 5 -10	50-100	200-300	~ 1-2	~3-6
	males	~ 8-25	0.03- 0.1			~ 1-2	~ 3-6	~ 3-10	~ 10-30
Human	follicular phase	20–150	0.1-0.5	10	0.03	0.1–1.5	< 5		
	mid-cycle phase	100–500	0.4-2	to	to	~ 1.5*	~ 5	0.3	1
	luteal phase	50–300	0.2-1	20	0.07	2–24	~ 8-80	to	to
	pregnancy 6/7 months	7,500-12,500	30-50	4,000-6000	15-20	60-90	200-300	1	3
	9 months	12,500-25,000	50-100	6,000-24,000	20-80	100-200	300-600		
	men	10–75	0.03-0.25	50-90	0.2-0.3	0.1–0.3	0.3-1	~3-10	~ 10-30

Figure 2. Range of corresponding plasma sex steroid concentrations in humans (156, 215, 430-434) and rodents (127, 152, 435, 436). *, 17-hydroxy-progesterone

MS, by acting on various immune and non-immune/neural systems as indicated below.

5. EFFECTS OF EXOGENOUS SEX STEROIDS IN MS AND EAE

5.1. 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 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.

5.2. 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.

Ref.	Strain	Gender	Model	Compound	Effect of compound on EAE			Efffect of compound on histopathologic or immunologic parameters
					onset	incidence	severity	
Treatment starting before or at time of immunization								
437	B10.RIII (H-2^r)	Femeles*	Active EAE	estradiol, proestrus levels	Delay			
				estradiol or estriol, pregnancy levels	Delay			
127	SJL	Females	Passive EAE	estriol, pregnancy levels	Delay	Decrease	Decrease	Increase in IL-10 secretion from activated splenocytes & in IgG1 seric levels (Th2 bias) Reduced neuroinflammation
438	B10.PL, Tg**	Females*	Active EAE	estradiol, estriol (various doses)	Delay	no Δ	Decrease	Decreased (trend) INFgamma production in activated splenocytes from (diestrus levels) estradiol treated group, as well as decrease in IgG1 and IgG2a serum levels
142	SJL, BP10.PL	Males , Femeles	Active EAE	estradiol, estriol (various doses)	Delay	(Decrease)	Reduced	Increase (trend) in IL-10 production in activated splenocytes from estriol treated mice. Decrease (trend) in INFgamma production in activated splenocytes drom estriol or estradiol treated mice. Less neuroinflammation and demyelination
128,129	C57BL/6	Females	Active EAE	estradiol, pregnancy levels	Delay	Decrease	Reduced/ no Δ***	Decrease in CNS recruitment of inflammatory cells Decreased frequency of TNFalpha producing T cells in the CNS or spleen and of TNFalpha producing CNS macrophages/microglia No Δ in Th1/Th2 cytokine profile from activated lymph node cells
131	C57BL/6	Males	Active EAE	estriol	no Δ	no Δ	Reduced	Decreased TNFalpha and INFgamma secretion and increased (only for males) IL-5 secretion in activated splenocytes
		Females	Active EAE	estriol	No EAE			
134		Females	Active EAE	estradiol, pregnancy levels				ERalpha crucial in protective effect
	C57BL/6		in wild type		Delay	Drop	Reduced	Decreased TNFalpha and INFgamma secretion
	B6.129 background		in ERalpha KO		Delay ****	no Δ	no Δ	from splenocytes
	B6.129 background		in ERbeta KO		Delay	Drop	Reduced	Less neuroinflammation
130	SJL	Females	Active EAE	EE, estradiol (pregnancy levels)	Delay	Drop	Reduced	Important increase in INFgamma secretion from activated splenocytes Decrease in IgG2a serum levels (but not IgG1) notably with EE Less neuroinflammation and demyelination
136	C57BL/6	Females (ovariectomized)	Active EAE	estradiol (diestrus or pregnancy dose)				ERalpha crucial in protective effect
			in wild type		No EAE			
			in ERalpha KO		no Δ	no Δ	no Δ	No Δ in TNFalpha or INFgamma secretion from CD4+ cells ****
			in ERbeta KO		No EAE			Less neuroinflammation
135	SJL	Females (ovariectomized)	Passive EAE	estradiol or ERalpha ligand, low dose	Delay	Decrease	Reduced	Reduction in Th1 but also Th2 cytokine profiles from activated spenocytes
132	C57BL/6	Females (ovariectomized)	Active EAE	estradiol or ERalpha ligand, low dose	(Delay)		Reduced	Reduction in TNFalpha and INFgamma secretion, increase in IL-5 secretion from activated splenocytes Less neuroinflammation. Less neuronal damage.
141	C57BL/6	Females (ovariectomized)	Active EAE	estradiol, ERalpha ligand	no EAE			same as ref. 132
				ERbeta ligand	no Δ		Reduced/ no Δ*****	No Δ in Th1/Th2 cytokine profiles from activated splenocytes No decrease in neuroinflammation. Less neuronal damage.
Treatment starting at or after disease onset								
				high/pregnancy levels				
127	SJL	Females	Passive EAE	estriol			Reduced	
142	SJL, B10.PL	Females	Active EAE	estradiol, estriol			not significant	
130	SJL	Females	Active EAE	EE			Reduced	

Figure 3. Effect of exogenous administration of natural estrogen or ethinyl-17alpha-estradiol (EE) on EAE in male or female mice on EAE development with respect to Th1/Th2 cytokine profiles and neurohistopathologic parameters. *, Earlier onset in ovariectomized mice; **, Transgenic mice bearing the functionally rearranged BV8S2 gene specific for MBP-Ac1-11 used as encephalitogen; ***, The estradiol treated mice showing clinical signs of disease reached the same level of severity as control mice; Possibly due to the slight increase in estradiol plasma levels; ****, Comparison using CD4+ cells (obtained from spleens of vehicle or low estradiol treated EAE mice) stimulated with syngeneic irradiated splenocytes in presence of encephalitogen; *****, Reduction after the acute initial phase.

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).

5.3. 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 (see Figure 1; 16, 17, 148-150). 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

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.

encounter in males) as well as DHT were found protective in all strains of (male or female) mice studied (21, 148-150, 152).

5.4. 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).

5.5. 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).

6. 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.

6.1. Overview of sex steroid signaling

6.1.1. 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 Figure 2).

6.1.2. 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).

6.1.3. 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).

6.2. Immune system

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

Estrogen 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).

6.2.1. 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 suspectiblity or severity, as mentionned 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 are 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 concomittant 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 controling 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 abscence of negative selection, potentially increasing the risk of autoimmunity on a long term (187, 254-256).

6.2.2. B cells

While chronic administration of high levels of estrogen or pregnancy suppress B lymphopoiesis, ovariectomy or orchidectomy induce B lymphopoiesis in the mouse bone marrow (252, 257, 258). B cells express intracellular but not membane 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 dose-dependent 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 progessive 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.

6.2.3. 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.

6.2.4 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 a 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 concomitent with a slight increase in circulating CD4+ IL-10+ 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.

6.3. Endothelial cells

Increasing evidence suggests that sex steroids may act by regulating the permeability of endothelial cells which compose the blood brain barrier, as in the periphery (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 and 320 for review). Acute treatment with pregnancy levels of estradiol has been shown to increase the expression of endothelial adhesion molecules, favoring leukocyte binding to endothelial cells, a first step in leukocyte entry into the parenchyma (321). In contrast, high and prolonged estrogen treatment 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. Indeed, estradiol has been reported to inhibit the migration of inflammatory cells in a rat carotid (artery) injury model (326). However, an *in vitro* study suggested that estrogen acts synergistically with myelin basic protein to cause mast cell infiltration into the brain parenchyma (288). Moreover, estrogen stimulates expression of brain derived neurotrophic factor (BDNF) which can induce 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, *in vivo*, in contrast to testosterone, estradiol reduces edema formation and ischemia- and vascular endothelial growth factor-induced blood-brain barrier disruption (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, 439). 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.

6.4. Glial cells

6.4.1. 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 (1-10nM) 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 of 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 are 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.

6.4.2. 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 also promotes remyelination and increases oligodendroglial cell maturation (47, 440). The interested reader is referred to the important revision on this subject by Schumacher *et al* (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*.

6.5. 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 degree of ERalpha or beta and AR expression is 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 the concept of 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 protects 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.

7. SAFETY CONSIDERATIONS OF SEX STEROID TREATMENTS

7.1. 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). Until now, HRT regimens were 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 disrupted 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.

7.2. Progestagens

It has 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.

8. 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.

9. ACKNOWLEDGEMENTS

Supported by UPMC and INSERM. The author would like to thank Drs S. Elkabes and C. Rohowsky-Kochan for critical comments, and Drs P. Kitabgi (UMR S 732), B. Fontaine and D. Pham -Dinh (UMR S 546) for their support. We apologize to those who published outstanding work related to this topic but were not cited here (including reports published after 2007).

10. REFERENCES

1. S. M. Gold, D. C. Mohr, I. Huitinga, P. Flachenecker, E. M. Sternberg and C. Heesen: The role of stress-response systems for the pathogenesis and progression of MS. Trends Immunol 26, 644-52 (2005)

2. A. M. Ercolini and S. D. Miller: Mechanisms of immunopathology in murine models of central nervous system demyelinating disease. J Immunol 176, 3293-8 (2006)

3. S. S. Zamvil and L. Steinman: Diverse targets for intervention during inflammatory and neurodegenerative phases of multiple sclerosis. Neuron 38, 685-8 (2003)

4. M. El Behi, S. Dubucquoi, D. Lefranc, H. Zephir, J. De Seze, P. Vermersch and L. Prin: New insights into cell responses involved in experimental autoimmune encephalomyelitis and multiple sclerosis. Immunol Lett 96, 11-26 (2005)

5. G. Krishnamoorthy, H. Lassmann, H. Wekerle and A. Holz: Spontaneous opticospinal encephalomyelitis in a double-transgenic mouse model of autoimmune T cell/B cell cooperation. J Clin Invest 116, 2385-92 (2006)

6. C. Pozzilli, V. Tomassini, F. Marinelli, A. Paolillo, C. Gasperini and S. Bastianello: 'Gender gap' in multiple sclerosis: magnetic resonance imaging evidence. Eur J Neurol 10, 95-7 (2003)

7. C. C. Whitacre, S. C. Reingold and P. A. O'Looney: A gender gap in autoimmunity. Science 283, 1277-8 (1999)

8. G. Cutter, R. Yadavalli, R. Marrie, T. Tyry, D. Campagnolo, B. Bullock and T. Vollmer: Changes in the Sex Ratio over Time in Multiple Sclerosis. In: 29th Annual Meeting American Academy of Neurology. (2007)

9. S. M. Orton, B. M. Herrera, I. M. Yee, W. Valdar, S. V. Ramagopalan, A. D. Sadovnick and G. C. Ebers: Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol 5, 932-6 (2006)

10. E. G. Celius and B. Vandvik: Multiple sclerosis in Oslo, Norway: prevalence on 1 January 1995 and incidence over a 25-year period. Eur J Neurol 8, 463-9 (2001)

11. C. W. Noonan, S. J. Kathman and M. C. White: Prevalence estimates for MS in the United States and evidence of an increasing trend for women. Neurology 58, 136-8 (2002)

12. M. Debouverie, S. Pittion-Vouyovitch, S. Louis, T. Roederer and F. Guillemin: Increasing incidence of multiple sclerosis among women in Lorraine, Eastern France. Mult Scler 13, 962-7 (2007)

13. A. B. Keith: Sex difference in Lewis rats in the incidence of recurrent experimental allergic encephalomyelitis. Nature 272, 824-5 (1978)

14. W. J. Trooster, A. W. Teelken, P. O. Gerrits, T. H. Lijnema, J. G. Loof, J. M. Minderhoud and P. Nieuwenhuis: The effect of gonadectomy on the clinical course of chronic experimental allergic encephalomyelitis. Clin Neurol Neurosurg 98, 222-6 (1996)

15. I. N. Montgomery and H. C. Rauch: Experimental allergic encephalomyelitis (EAE) in mice: primary control of EAE susceptibility is outside the H-2 complex. J Immunol 128, 421-5 (1982)

16. B. F. Bebo, Jr., A. A. Vandenbark and H. Offner: Male SJL mice do not relapse after induction of EAE with PLP 139-151. J Neurosci Res 45, 680-9 (1996)

17. B. F. Bebo, Jr., J. C. Schuster, A. A. Vandenbark and H. Offner: Gender differences in experimental autoimmune encephalomyelitis develop during the induction of the immune response to encephalitogenic peptides. J Neurosci Res 52, 420-6 (1998)

18. R. R. Voskuhl, H. Pitchekian-Halabi, A. MacKenzie-Graham, H. F. McFarland and C. S. Raine: Gender differences in autoimmune demyelination in the mouse: implications for multiple sclerosis. Ann Neurol 39, 724-33 (1996)

19. R. R. Voskuhl and K. Palaszynski: Sex hormones in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Neuroscientist 7, 258-70 (2001)

20. T. L. Papenfuss, C. J. Rogers, I. Gienapp, M. Yurrita, M. McClain, N. Damico, J. Valo, F. Song and C. C. Whitacre: Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. J Neuroimmunol 150, 59-69 (2004)

21. B. F. Bebo, Jr., E. Zelinka-Vincent, G. Adamus, D. Amundson, A. A. Vandenbark and H. Offner: Gonadal hormones influence the immune response to PLP 139-151 and the clinical course of relapsing experimental autoimmune encephalomyelitis. J Neuroimmunol 84, 122-30 (1998)

22. B. F. Bebo, Jr., J. C. Schuster, A. A. Vandenbark and H. Offner: Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. J Immunol 162, 35-40 (1999)

23. C. A. Kappel, R. W. Melvold and B. S. Kim: Influence of sex on susceptibility in the Theiler's murine encephalomyelitis virus model for multiple sclerosis. J Neuroimmunol 29, 15-9 (1990)

24. A. C. Fuller, B. Kang, H. K. Kang, H. Yahikozowa, M. C. Dal Canto and B. S. Kim: Gender bias in Theiler's virus-induced demyelinating disease correlates with the level of antiviral immune responses. J Immunol 175, 3955-63 (2005)

25. A. Fuller, H. Yahikozawa, E. Y. So, M. Dal Canto, C. S. Koh, C. J. Welsh and B. S. Kim: Castration of male C57L/J mice increases susceptibility and estrogen treatment restores resistance to Theiler's virus-induced demyelinating disease. J Neurosci Res 85, 871-81 (2007)

26. K. E. Hill, M. Pigmans, R. S. Fujinami and J. W. Rose: Gender variations in early Theiler's virus induced demyelinating disease: differential susceptibility and effects of IL-4, IL-10 and combined IL-4 with IL-10. J Neuroimmunol 85, 44-51 (1998)

27. C. Y. Yu and C. C. Whitacre: Sex, MHC and complement C4 in autoimmune diseases. Trends Immunol 25, 694-9 (2004)

28. R. J. Butterfield, E. P. Blankenhorn, R. J. Roper, J. F. Zachary, R. W. Doerge, J. Sudweeks, J. Rose and C. Teuscher: Genetic analysis of disease subtypes and sexual dimorphisms in mouse experimental allergic encephalomyelitis (EAE): relapsing/remitting and monophasic remitting/nonrelapsing EAE are immunogenetically distinct. J Immunol 162, 3096-102 (1999)

29. C. Teuscher, R. J. Butterfield, R. Z. Ma, J. F. Zachary, R. W. Doerge and E. P. Blankenhorn: Sequence polymorphisms in the chemokines Scya1 (TCA-3), Scya2 (monocyte chemoattractant protein (MCP)-1), and Scya12 (MCP-5) are candidates for eae7, a locus controlling susceptibility to monophasic remitting/nonrelapsing experimental allergic encephalomyelitis. J Immunol 163, 2262-6 (1999)

30. T. Vyshkina and B. Kalman: Haplotypes within genes of beta-chemokines in 17q11 are associated with multiple sclerosis: a second phase study. Hum Genet 118, 67-75 (2005)

31. P. D. Fillmore, E. P. Blankenhorn, J. F. Zachary and C. Teuscher: Adult gonadal hormones selectively regulate sexually dimorphic quantitative traits observed in experimental allergic encephalomyelitis. Am J Pathol 164, 167-75 (2004)

32. O. H. Kantarci, A. Goris, D. D. Hebrink, S. Heggarty, S. Cunningham, I. Alloza, E. J. Atkinson, M. de Andrade, C. T. McMurray, C. A. Graham, S. A. Hawkins, A. Billiau, B. Dubois, B. G. Weinshenker and K. Vandenbroeck: IFNG polymorphisms are associated with gender differences in susceptibility to multiple sclerosis. Genes Immun 6, 153-61 (2005)

33. O. H. Kantarci, L. F. Barcellos, E. J. Atkinson, P. P. Ramsay, R. Lincoln, S. J. Achenbach, M. De Andrade, S. L. Hauser and B. G. Weinshenker: Men transmit MS more often to their children vs women: the Carter effect. Neurology 67, 305-10 (2006)

34. K. M. Palaszynski, D. L. Smith, S. Kamrava, P. S. Burgoyne, A. P. Arnold and R. R. Voskuhl: A yin-yang effect between sex chromosome complement and sex hormones on the immune response. Endocrinology 146, 3280-5 (2005)

35. C. Teuscher, R. Noubade, K. Spach, B. McElvany, J. Y. Bunn, P. D. Fillmore, J. F. Zachary and E. P. Blankenhorn: Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A 103, 8024-9 (2006)

36. M. Niino, S. Kikuchi, T. Fukazawa, I. Yabe and K. Tashiro: Estrogen receptor gene polymorphism in Japanese patients with multiple sclerosis. J Neurol Sci 179 (S 1-2), 70-5 (2000)

37. S. Kikuchi, T. Fukazawa, M. Niino, I. Yabe, R. Miyagishi, T. Hamada and K. Tashiro: Estrogen receptor gene polymorphism and multiple sclerosis in Japanese patients: interaction with HLA-DRB1*1501 and disease modulation. J Neuroimmunol 128, 77-81 (2002)

38. G. Savettieri, R. Cittadella, P. Valentino, I. Manna, V. Andreoli, A. La Russa, G. La Porta, F. Ruscica, P. Ragonese, D. Pirritano, S. Bonavita, G. Tedeschi and A. Quattrone: Lack of association between estrogen receptor 1 gene polymorphisms and multiple sclerosis in southern Italy in humans. Neurosci Lett 327, 115-8 (2002)

39. W. Davies and L. S. Wilkinson: It is not all hormones: alternative explanations for sexual differentiation of the brain. Brain Res 1126, 36-45 (2006)

40. D. K. Nguyen and C. M. Disteche: Dosage compensation of the active X chromosome in mammals. Nat Genet 38, 47-53 (2006)

41. J. H. Gilmore, W. Lin, M. W. Prastawa, C. B. Looney, Y. S. Vetsa, R. C. Knickmeyer, D. D. Evans, J. K. Smith, R. M. Hamer, J. A. Lieberman and G. Gerig: Regional gray matter growth, sexual dimorphism, and cerebral asymmetry in the neonatal brain. J Neurosci 27, 1255-60 (2007)

42. M. Cerghet, R. P. Skoff, D. Bessert, Z. Zhang, C. Mullins and M. S. Ghandour: Proliferation and death of oligodendrocytes and myelin proteins are differentially regulated in male and female rodents. J Neurosci 26, 1439-47 (2006)

43. J. S. Peper, R. M. Brouwer, D. I. Boomsma, R. S. Kahn and H. E. Hulshoff Pol: Genetic influences on human brain

structure: a review of brain imaging studies in twins. Hum Brain Mapp 28, 464-73 (2007)

44. P. A. Filipek, C. Richelme, D. N. Kennedy and V. S. Caviness, Jr.: The young adult human brain: an MRI-based morphometric analysis. Cereb Cortex 4, 344-60 (1994)

45. J. M. Goldstein, L. J. Seidman, N. J. Horton, N. Makris, D. N. Kennedy, V. S. Caviness, Jr., S. V. Faraone and M. T. Tsuang: Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb Cortex 11, 490-7 (2001)

46. D. L. Greenberg, M. E. Payne, J. R. MacFall, J. M. Provenzale, D. C. Steffens and R. R. Krishnan: Differences in brain volumes among males and female hormone-therapy users and nonusers. Psychiatry Res 147, 127-34 (2006)

47. M. Marin-Husstege, M. Muggironi, D. Raban, R. P. Skoff and P. Casaccia-Bonnefil: Oligodendrocyte progenitor proliferation and maturation is differentially regulated by male and female sex steroid hormones. Dev Neurosci 26, 245-54 (2004)

48. M. C. Morale, F. Gallo, C. Tirolo, N. Testa, S. Caniglia, N. Marletta, V. Spina-Purrello, R. Avola, F. Caucci, P. Tomasi, G. Delitala, N. Barden and B. Marchetti: Neuroendocrine-immune (NEI) circuitry from neuron-glial interactions to function: Focus on gender and HPA-HPG interactions on early programming of the NEI system. Immunol Cell Biol 79, 400-17 (2001)

49. C. A. Roca, P. J. Schmidt, P. A. Deuster, M. A. Danaceau, M. Altemus, K. Putnam, G. P. Chrousos, L. K. Nieman and D. R. Rubinow: Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. J Clin Endocrinol Metab 90, 4224-31 (2005)

50. I. C. Weiss, C. R. Pryce, A. L. Jongen-Relo, N. I. Nanz-Bahr and J. Feldon: Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 152, 279-95 (2004)

51. M. Uhart, R. Y. Chong, L. Oswald, P. I. Lin and G. S. Wand: Gender differences in hypothalamic-pituitary-adrenal (HPA) axis reactivity. Psychoneuroendocrinology 31, 642-52 (2006)

52. F. Homo-Delarche, F. Fitzpatrick, N. Christeff, E. A. Nunez, J. F. Bach and M. Dardenne: Sex steroids, glucocorticoids, stress and autoimmunity. J Steroid Biochem Mol Biol 40, 619-37 (1991)

53. A. C. Griffin and C. C. Whitacre: Sex and strain differences in the circadian rhythm fluctuation of endocrine and immune function in the rat: implications for rodent models of autoimmune disease. J Neuroimmunol 35, 53-64 (1991)

54. V. K. Patchev, S. Hayashi, C. Orikasa and O. F. Almeida: Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. Faseb J 9, 419-23 (1995)

55. I. J. Elenkov and G. P. Chrousos: Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. Trends Endocrinol Metab 10, 359-368 (1999)

56. D. C. Mohr, S. L. Hart, L. Julian, D. Cox and D. Pelletier: Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. BMJ, 328, 731 (2004)

57. A. C. Griffin, W. D. Lo, A. C. Wolny and C. C. Whitacre: Suppression of experimental autoimmune encephalomyelitis by restraint stress: sex differences. J Neuroimmunol 44, 103-16 (1993)

58. J. Alley, S. Khasabov, D. Simone, A. Beitz, M. Rodriguez and M. K. Njenga: More severe neurologic deficits in SJL/J male than female mice following Theiler's virus-induced CNS demyelination. Exp Neurol 180, 14-24 (2003)

59. A. N. Sieve, A. J. Steelman, C. R. Young, R. Storts, T. H. Welsh, C. J. Welsh and M. W. Meagher: Chronic restraint stress during early Theiler's virus infection exacerbates the subsequent demyelinating disease in SJL mice. J Neuroimmunol 155, 103-18 (2004)

60. L. M. van Winsen, D. F. Muris, C. H. Polman, C. D. Dijkstra, T. K. van den Berg and B. M. Uitdehaag: Sensitivity to glucocorticoids is decreased in relapsing remitting multiple sclerosis. J Clin Endocrinol Metab 90, 734-40 (2005)

61. R. Avitsur, J. L. Stark, F. S. Dhabhar, D. A. Padgett and J. F. Sheridan: Social disruption-induced glucocorticoid resistance: kinetics and site specificity. J Neuroimmunol 124, 54-61 (2002)

62. B. Marchetti, M. C. Morale, N. Testa, C. Tirolo, S. Caniglia, S. Amor, C. D. Dijkstra and N. Barden: Stress, the immune system and vulnerability to degenerative disorders of the central nervous system in transgenic mice expressing glucocorticoid receptor antisense RNA. Brain Res Brain Res Rev 37, 259-72 (2001)

63. C. Pelfrey: Sexual dimorphism in autoimmunity: a focus on Th1/Th2 cytokines and multiple sclerosis. Clin Appl Immunol Rev 1, 331-345 (2001)

64. C. C. Whitacre: Sex differences in autoimmune disease. Nat Immunol, 2 (9), 777-80 (2001)

65. R. Smith and J. W. Studd: A pilot study of the effect upon multiple sclerosis of the menopause, hormone replacement therapy and the menstrual cycle. J R Soc Med, 85, 612-3 (1992)

66. M. K. Houtchens, N. Gregori and J. W. Rose: Understanding fluctuations of multiple sclerosis across the menstrual Cycle. Int J MS Care 2, 7-14 (2000)

67. A. Zorgdrager and J. De Keyser: The premenstrual period and exacerbations in multiple sclerosis. Eur Neurol 48, 204-6 (2002)

68. P. Holmqvist, M. Wallberg, M. Hammar, A. M. Landtblom and J. Brynhildsen: Symptoms of multiple sclerosis in women in relation to sex steroid exposure. Maturitas 54, 149-53 (2006)

69. V. Tomassini, E. Onesti, C. Mainero, E. Giugni, A. Paolillo, M. Salvetti, F. Nicoletti and C. Pozzilli: Sex hormones modulate brain damage in multiple sclerosis: MRI evidence. J Neurol Neurosurg Psychiatry 76, 272-5 (2005)

70. S. Bansil, H. J. Lee, S. Jindal, C. R. Holtz and S. D. Cook: Correlation between sex hormones and magnetic resonance imaging lesions in multiple sclerosis. Acta Neurol Scand 99, 91-4 (1999)

71. C. Pozzilli, P. Falaschi, C. Mainero, A. Martocchia, R. D'Urso, A. Proietti, M. Frontoni, S. Bastianello and M. Filippi: MRI in multiple sclerosis during the menstrual cycle: relationship with sex hormone patterns. Neurology 53, 622-4 (1999)

72. M. Rovaris and M. Filippi: Magnetic resonance techniques to monitor disease evolution and treatment trial outcomes in multiple sclerosis. Curr Opin Neurol 12, 337-44 (1999)

73. M. Filippi, M. A. Rocca and G. Comi: The use of quantitative magnetic-resonance-based techniques to monitor the evolution of multiple sclerosis. Lancet Neurol 2, 337-46 (2003)

74. R. Zivadinov and T. P. Leist: Clinical-magnetic resonance imaging correlations in multiple sclerosis. J Neuroimaging 15 (4 Suppl), 10S-21S (2005)

75. R. Zivadinov and R. Bakshi: Role of MRI in multiple sclerosis I: inflammation and lesions. Front Biosci 9, 665-83 (2004)

76. R. Zivadinov and R. Bakshi: Role of MRI in multiple sclerosis II: brain and spinal cord atrophy. Front Biosci 9, 647-64 (2004)

77. D. R. Maccio, G. Calfa, M. Volosin and G. A. Roth: Serum testosterone and corticosterone levels in acute experimental autoimmune encephalomyelitis (EAE) in male Wistar rats. Neuro Endocrinol Lett 25, 196-200 (2004)

78. S. C. Foster, C. Daniels, D. N. Bourdette and B. F. Bebo, Jr.: Dysregulation of the hypothalamic-pituitary-gonadal axis in experimental autoimmune encephalomyelitis and multiple sclerosis. J Neuroimmunol 140, 78-87 (2003)

79. Y. Deri, A. Katzav, J. Chapman and A. Biegon: Sex differences and estrus cycle effects in experimental autoimmune encephalomyelitis (EAE) in mice. Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience. Online (2006).

80. E. V. Simon, I. Topalli, A. Touray and S.A. Sadiq: Decreased Serum Testosterone Levels in Multiple Sclerosis. Neurology 66 (Suppl. 2), A225 (2006)

81. A. B. Keith: Effect of pregnancy on experimental allergic encephalomyelitis in guinea pigs and rats. J Neurol Sci 38, 317-26 (1978)

82. T. Brenner, S. Evron and O. Abramsky: Effect of experimental autoimmune encephalomyelitis on pregnancy: studies in rabbits and rats. Isr J Med Sci 27, 181-5 (1991)

83. L. A. Mertin and V. M. Rumjanek: Pregnancy and the susceptibility of Lewis rats to experimental allergic encephalomyelitis. J Neurol Sci 68, 15-24 (1985)

84a. A. Langer-Gould, H. Garren, A. Slansky, P. J. Ruiz and L. Steinman: Late pregnancy suppresses relapses in experimental autoimmune encephalomyelitis: evidence for a suppressive pregnancy-related serum factor. J Immunol 169, 1084-91 (2002)

84b. M. A. McClain, N. N. Gatson, N. D. Powell, T. L. Papenfuss, I. E. Gienapp, F. Song, T. M. Shawler, A. Kithcart and C. C. Whitacre: Pregnancy Suppresses Experimental Autoimmune Encephalomyelitis through Immunoregulatory Cytokine Production. J Immunol 179, 8146-52 (2007).

85. B. Runmarker and O. Andersen: Pregnancy is associated with a lower risk of onset and a better prognosis in multiple sclerosis. Brain 118, 253-61 (1995)

86. S. Vukusic, M. Hutchinson, M. Hours, T. Moreau, P. Cortinovis-Tourniaire, P. Adeleine, C. Confavreux and The Pregnancy In Multiple Sclerosis Group: Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain 127, 1353-60 (2004)

87. I. J. Elenkov, R. L. Wilder, V. K. Bakalov, A. A. Link, M. A. Dimitrov, S. Fisher, M. Crane, K. S. Kanik and G. P. Chrousos: IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. J Clin Endocrinol Metab 86, 4933-8 (2001)

88. M. A. van Walderveen, M. W. Tas, F. Barkhof, C. H. Polman, S. T. Frequin, O. R. Hommes and J. Valk: Magnetic resonance evaluation of disease activity

during pregnancy in multiple sclerosis. Neurology 44, 327-9 (1994)

89. M. J. Polanczyk, C. Hopke, J. Huan, A. A. Vandenbark and H. Offner: Enhanced FoxP3 expression and Treg cell function in pregnant and estrogen-treated mice. J Neuroimmunol 170, 85-92 (2005)

90. S. Sanchez-Ramon, A. J. Navarro, C. Aristimuno, M. Rodriguez-Mahou, J. M. Bellon, E. Fernandez-Cruz and C. de Andres: Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity. Immunol Lett 96, 195-201 (2005)

91. M. Saraste, S. Vaisanen, A. Alanen and L. Airas: Clinical and immunologic evaluation of women with multiple sclerosis during and after pregnancy. Gend Med 4, 45-55 (2007)

92. D. J. Cua, D. R. Hinton and S. A. Stohlman: Self-antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice. Th2-mediated suppression of autoimmune disease. J Immunol 155, 4052-9 (1995)

93. R. Kumar, W. R. Cohen, P. Silva and F. H. Epstein: Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. J Clin Invest 63, 342-4 (1979)

94. M. T. Cantorna and B. D. Mahon: Mounting evidence for vitamin D as an environmental factor affecting autoimmune disease prevalence. Exp Biol Med (Maywood) 229, 1136-42 (2004)

95. Y. Arnson, H. Amital and Y. Shoenfeld: Vitamin D and autoimmunity: new aetiological and therapeutic considerations. Ann Rheum Dis 66, 1137-1142 (2007)

96. L. Yang, Y. Hu, X. Li, J. Zhao and Y. Hou: Prolactin modulates the functions of murine spleen CD11c-positive dendritic cells. Int Immunopharmacol 6, 1478-86 (2006)

97. L. M. Nelson, G. M. Franklin and M. C. Jones: Risk of multiple sclerosis exacerbation during pregnancy and breast-feeding. Jama 259, 3441-3 (1988)

98. C. Confavreux, M. Hutchinson, M. M. Hours, P. Cortinovis-Tourniaire, T. Moreau and the Pregnancy Multiple Sclerosis Group: Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. N Engl J Med 339, 285-91 (1998)

99. L. Grinsted, A. Heltberg, C. Hagen and H. Djursing: Serum sex hormone and gonadotropin concentrations in premenopausal women with multiple sclerosis. J Intern Med 226, 241-4 (1989)

100. J. Kira, M. Harada, Y. Yamaguchi, N. Shida and I. Goto: Hyperprolactinemia in multiple sclerosis. J Neurol Sci 102, 61-6 (1991)

101. S. T. Azar and B. Yamout: Prolactin secretion is increased in patients with multiple sclerosis. Endocr Res, 25 (2), 207-14 (1999)

102. C. Gregg, V. Shikar, P. Larsen, G. Mak, A. Chojnacki, V. W. Yong and S. Weiss: White matter plasticity and enhanced remyelination in the maternal CNS. J Neurosci 27, 1812-23 (2007)

103. H. Bohn: (Detection and characterization of pregnancy proteins in the human placenta and their quantitative immunochemical determination in sera from pregnant women). Arch Gynakol 210, 440-57 (1971)

104. T. M. Lin, S. P. Halbert and W. N. Spellacy: Measurement of pregnancy-associated plasma proteins during human gestation. J Clin Invest 54, 576-82 (1974)

105. S. H. Um, C. Mulhall, A. Alisa, A. R. Ives, J. Karani, R. Williams, A. Bertoletti and S. Behboudi: Alpha-fetoprotein impairs APC function and induces their apoptosis. J Immunol 173, 1772-8 (2004)

106. F. P. Noonan, W. J. Halliday, H. Morton and G. J. Clunie: Early pregnancy factor is immunosuppressive. Nature 278, 649-51 (1979)

107. T. Dschietzig, C. Bartsch, M. Greinwald, G. Baumann and K. Stangl: The pregnancy hormone relaxin binds to and activates the human glucocorticoid receptor. Ann N Y Acad Sci 1041, 256-71 (2005)

108. C. T. Ha, R. Waterhouse, J. Wessells, J. A. Wu and G. S. Dveksler: Binding of pregnancy-specific glycoprotein 17 to CD9 on macrophages induces secretion of IL-10, IL-6, PGE2, and TGF-beta1. J Leukoc Biol 77, 948-57 (2005)

109. M. Irony-Tur-Sinai, N. Grigoriadis, A. Lourbopoulos, F. Pinto-Maaravi, O. Abramsky and T. Brenner: Amelioration of autoimmune neuroinflammation by recombinant human alpha-fetoprotein. Exp Neurol 198, 136-44 (2006)

110. K. Santora, C. Rasa, D. Visco, B. G. Steinetz and C. A. Bagnell: Antiarthritic effects of relaxin, in combination with estrogen, in rat adjuvant-induced arthritis. J Pharmacol Exp Ther 322, 887-93 (2007)

111. Y. C. Zang, J. B. Halder, J. Hong, V. M. Rivera and J. Z. Zhang: Regulatory effects of estriol on T cell migration and cytokine profile: inhibition of transcription factor NF-kappa B. J Neuroimmunol 124, 106-14 (2002)

112. P. J. Havel, S. Kasim-Karakas, G. R. Dubuc, W. Mueller and S. D. Phinney: Gender differences in plasma leptin concentrations. Nat Med, 2 (9), 949-50 (1996)

113. M. Rosenbaum, M. Nicolson, J. Hirsch, S. B. Heymsfield, D. Gallagher, F. Chu and R. L. Leibel: Effects of gender, body composition, and menopause on plasma

concentrations of leptin. J Clin Endocrinol Metab 81, 3424-7 (1996)

114. M. F. Saad, S. Damani, R. L. Gingerich, M. G. Riad-Gabriel, A. Khan, R. Boyadjian, S. D. Jinagouda, K. el-Tawil, R. K. Rude and V. Kamdar: Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab 82, 579-84 (1997)

115. J. M. Elbers, H. Asscheman, J. C. Seidell, M. Frolich, A. E. Meinders and L. J. Gooren: Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. J Clin Endocrinol Metab 82, 3267-70 (1997)

116. V. Sanna, A. Di Giacomo, A. La Cava, R. I. Lechler, S. Fontana, S. Zappacosta and G. Matarese: Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest 111, 241-50 (2003)

117. V. De Rosa, C. Procaccini, G. Cali, G. Pirozzi, S. Fontana, S. Zappacosta, A. La Cava and G. Matarese: A key role of leptin in the control of regulatory T cell proliferation. Immunity 26, 241-55 (2007)

118. G. M. Lord, G. Matarese, J. K. Howard, R. J. Baker, S. R. Bloom and R. I. Lechler: Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394, 897-901 (1998)

119. G. Matarese, P. B. Carrieri, A. La Cava, F. Perna, V. Sanna, V. De Rosa, D. Aufiero, S. Fontana and S. Zappacosta: Leptin increase in multiple sclerosis associates with reduced number of CD4+CD25+ regulatory T cells. Proc Natl Acad Sci U S A 102, 5150-5 (2005)

120. V. De Rosa, C. Procaccini, A. La Cava, P. Chieffi, G. F. Nicoletti, S. Fontana, S. Zappacosta and G. Matarese: Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis. J Clin Invest 116, 447-55 (2006)

121. M. A. Hernan, M. J. Hohol, M. J. Olek, D. Spiegelman and A. Ascherio: Oral contraceptives and the incidence of multiple sclerosis. Neurology 55, 848-54 (2000)

122. L. Villard-Mackintosh and M. P. Vessey: Oral contraceptives and reproductive factors in multiple sclerosis incidence. Contraception 47, 161-8 (1993)

123. M. Thorogood and P. C. Hannaford: The influence of oral contraceptives on the risk of multiple sclerosis. Br J Obstet Gynaecol 105, 1296-9 (1998)

124. A. Alonso, S. S. Jick, M. J. Olek, A. Ascherio, H. Jick and M. A. Hernan: Recent use of oral contraceptives and the risk of multiple sclerosis. Arch Neurol 62, 1362-5 (2005)

125. S. Poser, N. E. Raun, J. Wikstrom and W. Poser: Pregnancy, oral contraceptives and multiple sclerosis. Acta Neurol Scand 59, 108-18 (1979)

126. H. Offner: Neuroimmunoprotective effects of estrogen and derivatives in experimental autoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. J Neurosci Res 78, 603-24 (2004)

127. S. Kim, S. M. Liva, M. A. Dalal, M. A. Verity and R. R. Voskuhl: Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology 52, 1230-8 (1999)

128. A. Ito, B. F. Bebo, Jr., A. Matejuk, A. Zamora, M. Silverman, A. Fyfe-Johnson and H. Offner: Estrogen treatment down-regulates TNF-alpha production and reduces the severity of experimental autoimmune encephalomyelitis in cytokine knockout mice. J Immunol 167, 542-52 (2001)

129. A. Ito, A. C. Buenafe, A. Matejuk, A. Zamora, M. Silverman, J. Dwyer, A. A. Vandenbark and H. Offner: Estrogen inhibits systemic T cell expression of TNF-alpha and recruitment of TNF-alpha(+) T cells and macrophages into the CNS of mice developing experimental encephalomyelitis. Clin Immunol 102, 275-82 (2002)

130. S. Subramanian, A. Matejuk, A. Zamora, A. A. Vandenbark and H. Offner: Oral feeding with ethinyl estradiol suppresses and treats experimental autoimmune encephalomyelitis in SJL mice and inhibits the recruitment of inflammatory cells into the central nervous system. J Immunol 170, 1548-55 (2003)

131. K. M. Palaszynski, H. Liu, K. K. Loo and R. R. Voskuhl: Estriol treatment ameliorates disease in males with experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neuroimmunol 149, 84-9 (2004)

132. L. B. Morales, K. K. Loo, H. B. Liu, C. Peterson, S. Tiwari-Woodruff and R. R. Voskuhl: Treatment with an estrogen receptor alpha ligand is neuroprotective in experimental autoimmune encephalomyelitis. J Neurosci 26, 6823-33 (2006)

133. H. B. Liu, K. K. Loo, K. Palaszynski, J. Ashouri, D. B. Lubahn and R. R. Voskuhl: Estrogen receptor alpha mediates estrogen's immune protection in autoimmune disease. J Immunol 171, 6936-40 (2003)

134. M. Polanczyk, A. Zamora, S. Subramanian, A. Matejuk, D. L. Hess, E. P. Blankenhorn, C. Teuscher, A. A. Vandenbark and H. Offner: The protective effect of 17beta-estradiol on experimental autoimmune encephalomyelitis is mediated through estrogen receptor-alpha. Am J Pathol 163, 1599-605 (2003)

135. M. M. Elloso, K. Phiel, R. A. Henderson, H. A. Harris and S. J. Adelman: Suppression of experimental autoimmune encephalomyelitis using estrogen receptor-selective ligands. J Endocrinol 185, 243-52 (2005)

136. L. Garidou, S. Laffont, V. Douin-Echinard, C. Coureau, A. Krust, P. Chambon and J. C. Guery: Estrogen receptor alpha signaling in inflammatory leukocytes is dispensable for 17beta-estradiol-mediated inhibition of experimental autoimmune encephalomyelitis. J Immunol 173, 2435-42 (2004)

137. M. J. Polanczyk, R. E. Jones, S. Subramanian, M. Afentoulis, C. Rich, M. Zakroczymski, P. Cooke, A. A. Vandenbark and H. Offner: T lymphocytes do not directly mediate the protective effect of estrogen on experimental autoimmune encephalomyelitis. Am J Pathol 165, 2069-77 (2004)

138. C. M. Pelfrey, I. R. Moldovan, A. C. Cotleur, N. Zamor and R. A. Rudick: Effects of sex hormones on costimulatory molecule expression in multiple sclerosis. J Neuroimmunol 167, 190-203 (2005)

139. M. Polanczyk, S. Yellayi, A. Zamora, S. Subramanian, M. Tovey, A. A. Vandenbark, H. Offner, J. F. Zachary, P. D. Fillmore, E. P. Blankenhorn, J. A. Gustafsson and C. Teuscher: Estrogen receptor-1 (Esr1) and -2 (Esr2) regulate the severity of clinical experimental allergic encephalomyelitis in male mice. Am J Pathol 164, 1915-24 (2004)

140. R. A. Jarred, S. J. McPherson, J. J. Bianco, J. F. Couse, K. S. Korach and G. P. Risbridger: Prostate phenotypes in estrogen-modulated transgenic mice. Trends Endocrinol Metab 13, 163-8 (2002)

141. S. Tiwari-Woodruff, L. B. Morales, R. Lee and R. R. Voskuhl: Differential neuroprotective and antiinflammatory effects of estrogen receptor (ER)alpha and ERbeta ligand treatment. Proc Natl Acad Sci U S A 104, 14813-8 (2007)

142. B. F. Bebo, Jr., A. Fyfe-Johnson, K. Adlard, A. G. Beam, A. A. Vandenbark and H. Offner: Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. J Immunol 166, 2080-9 (2001)

143. B. T. Zhu, G. Z. Han, J. Y. Shim, Y. Wen and X. R. Jiang: Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: Insights into the structural determinants favoring a differential subtype binding. Endocrinology 147, 4132-50 (2006)

144. M. M. Cherrier, A. M. Matsumoto, J. K. Amory, S. Ahmed, W. Bremner, E. R. Peskind, M. A. Raskind, M. Johnson and S. Craft: The role of aromatization in testosterone supplementation: effects on cognition in older men. Neurology 64, 290-6 (2005)

145. A. Vottero, V. Rochira, M. Capelletti, I. Viani, L. Zirilli, T. M. Neri, C. Carani, S. Bernasconi and L. Ghizzoni: Aromatase is differentially expressed in peripheral blood leukocytes from children, and adult female and male subjects. Eur J Endocrinol 154, 425-31 (2006)

146. T. R. Pak, W. C. Chung, T. D. Lund, L. R. Hinds, C. M. Clay and R. J. Handa: The androgen metabolite, 5alpha-androstane-3beta, 17beta-diol, is a potent modulator of estrogen receptor-beta1-mediated gene transcription in neuronal cells. Endocrinology 146, 147-55 (2005)

147. T. R. Pak, W. C. Chung, L. R. Hinds and R. J. Handa: Estrogen receptor-beta mediates dihydrotestosterone-induced stimulation of the arginine vasopressin promoter in neuronal cells. Endocrinology 148, 3371-82 (2007)

148. M. Dalal, S. Kim and R. R. Voskuhl: Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. J Immunol 159, 3-6 (1997)

149. M. E. Smith, N. L. Eller, H. F. McFarland, M. K. Racke and C. S. Raine: Age dependence of clinical and pathological manifestations of autoimmune demyelination. Implications for multiple sclerosis. Am J Pathol 155, 1147-61 (1999)

150. K. M. Palaszynski, K. K. Loo, J. F. Ashouri, H. B. Liu and R. R. Voskuhl: Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neuroimmunol 146, 144-52 (2004)

151. S. M. Liva and R. R. Voskuhl: Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. J Immunol 167, 2060-7 (2001)

152. F. Macrides, A. Bartke and S. Dalterio: Strange females increase plasma testosterone levels in male mice. Science 189, 1104-6 (1975)

153. B. G. Arnason and D. P. Richman: Effect of oral contraceptives on experimental demyelinating disease. Arch Neurol 21, 103-8 (1969)

154. G. E. Hoffman, W. W. Le, A. Z. Murphy and C. L. Koski: Divergent effects of ovarian steroids on neuronal survival during experimental allergic encephalitis in Lewis rats. Exp Neurol 171, 272-84 (2001)

155. G. A. Elliott, A. J. Gibbons and M. E. Greig: A comparison of the effects of melengestrol acetate with a combination of hydrocortisone acetate and medroxyprogesterone acetate and with other steroids in the treatment of experimental alleric encephalomyelitis in Wistar rats. Acta Neuropathol (Berl) 23, 95-104 (1973)

156. N. L. Sicotte, S. M. Liva, R. Klutch, P. Pfeiffer, S. Bouvier, S. Odesa, T. C. Wu and R. R. Voskuhl: Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol 52, 421-8 (2002)

157. S. Vukusic and C. Confavreux: Pregnancy and multiple sclerosis: the children of PRIMS. Clin Neurol Neurosurg 108, 266-70 (2006)

158. N. L. Sicotte, B. S. Giesser, V. Tandon, R. Klutch, B. Steiner, A. E. Drain, D. W. Shattuck, L. Hull, H. J. Wang, R. M. Elashoff, R. S. Swerdloff and R. R. Voskuhl: Testosterone treatment in multiple sclerosis: a pilot study. Arch Neurol 64, 683-8 (2007)

159. S. Nilsson and J. A. Gustafsson: Biological role of estrogen and estrogen receptors. Crit Rev Biochem Mol Biol 37, 1-28 (2002)

160. H. S. Fox, B. L. Bond and T. G. Parslow: Estrogen regulates the IFN-gamma promoter. J Immunol 146, 4362-7 (1991)

161. L. I. McKay and J. A. Cidlowski: Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocr Rev 20, 435-59 (1999)

162. N. Vasudevan and D. W. Pfaff: Membrane-initiated actions of estrogens in neuroendocrinology: emerging principles. Endocr Rev 28, 1-19 (2007)

163. M. Razandi, A. Pedram, G. L. Greene and E. R. Levin: Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERalpha and ERbeta expressed in Chinese hamster ovary cells. Mol Endocrinol 13, 307-19 (1999)

164. C. B. Wade, S. Robinson, R. A. Shapiro and D. M. Dorsa: Estrogen receptor (ER)alpha and ERbeta exhibit unique pharmacologic properties when coupled to activation of the mitogen-activated protein kinase pathway. Endocrinology 142, 2336-42 (2001)

165. C. Stirone, S. P. Duckles, D. N. Krause and V. Procaccio: Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. Mol Pharmacol 68, 959-65 (2005)

166. S. H. Yang, R. Liu, E. J. Perez, Y. Wen, S. M. Stevens, Jr., T. Valencia, A. M. Brun-Zinkernagel, L. Prokai, Y. Will, J. Dykens, P. Koulen and J. W. Simpkins: Mitochondrial localization of estrogen receptor beta. Proc Natl Acad Sci U S A 101, 4130-5 (2004)

167. C. V. Demonacos, N. Karayanni, E. Hatzoglou, C. Tsiriyiotis, D. A. Spandidos and C. E. Sekeris: Mitochondrial genes as sites of primary action of steroid hormones. Steroids 61, 226-32 (1996)

168. K. Strehlow, S. Rotter, S. Wassmann, O. Adam, C. Grohe, K. Laufs, M. Bohm and G. Nickenig: Modulation of antioxidant enzyme expression and function by estrogen. Circ Res 93, 170-7 (2003)

169. J. D. Yager and J. Q. Chen: Mitochondrial estrogen receptors--new insights into specific functions. Trends Endocrinol Metab 18, 89-91 (2007)

170. C. M. Revankar, D. F. Cimino, L. A. Sklar, J. B. Arterburn and E. R. Prossnitz: A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307, 1625-30 (2005)

171. C. M. Revankar, H. D. Mitchell, A. S. Field, R. Burai, C. Corona, C. Ramesh, L. A. Sklar, J. B. Arterburn and E. R. Prossnitz: Synthetic estrogen derivatives demonstrate the functionality of intracellular GPR30. ACS Chem Biol 2, 536-44 (2007)

172. C. D. Toran-Allerand, X. Guan, N. J. MacLusky, T. L. Horvath, S. Diano, M. Singh, E. S. Connolly, Jr., I. S. Nethrapalli and A. A. Tinnikov: ER-X: a novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. J Neurosci 22, 8391-401 (2002)

173. C. Owman, C. Nilsson and S. J. Lolait: Cloning of cDNA encoding a putative chemoattractant receptor. Genomics 37, 187-94 (1996)

174. A. D. Mooradian: Antioxidant properties of steroids. J Steroid Biochem Mol Biol 45, 509-11 (1993)

175. L. Prokai, K. Prokai-Tatrai, P. Perjesi, A. D. Zharikova, E. J. Perez, R. Liu and J. W. Simpkins: Quinol-based cyclic antioxidant mechanism in estrogen neuroprotection. Proc Natl Acad Sci U S A 100, 11741-6 (2003)

176. D. Manthey and C. Behl: From structural biochemistry to expression profiling: neuroprotective activities of estrogen. Neuroscience 138, 845-50 (2006)

177. Y. Numakawa, T. Matsumoto, D. Yokomaku, T. Taguchi, E. Niki, H. Hatanaka, H. Kunugi and T. Numakawa: 17beta-estradiol protects cortical neurons against oxidative stress-induced cell death through reduction in the activity of mitogen-activated protein kinase and in the accumulation of intracellular calcium. Endocrinology 148, 627-37 (2007)

178. N. Santanam, R. Shern-Brewer, R. McClatchey, P. Z. Castellano, A. A. Murphy, S. Voelkel and S. Parthasarathy: Estradiol as an antioxidant: incompatible with its physiological concentrations and function. J Lipid Res 39, 2111-8 (1998)

179. J. W. Simpkins and J. A. Dykens: Mitochondrial mechanisms of estrogen neuroprotection. Brain Res Rev 57, 421-30 (2008)

180. C. Borras, J. Gambini and J. Vina: Mitochondrial oxidant generation is involved in determining why females live longer than males. Front Biosci 12, 1008-13 (2007)

181. N. Z. Lu, S. E. Wardell, K. L. Burnstein, D. Defranco, P. J. Fuller, V. Giguere, R. B. Hochberg, L. McKay, J. M. Renoir, N. L. Weigel, E. M. Wilson, D. P. McDonnell and J. A. Cidlowski: International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid,

progesterone, and androgen receptors. Pharmacol Rev 58, 782-97 (2006)

182. M. Schumacher, R. Guennoun, A. Ghoumari, C. Massaad, F. Robert, M. El-Etr, Y. Akwa, K. Rajkowski and E. E. Baulieu: Novel perspectives for progesterone in hormone replacement therapy, with special reference to the nervous system. Endocr Rev 28, 387-439 (2007)

183. T. V. Nguyen, M. Yao and C. J. Pike: Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. J Neurochem 94, 1639-51 (2005)

184. T. V. Nguyen, M. Yao and C. J. Pike: Flutamide and cyproterone acetate exert agonist effects: induction of androgen receptor-dependent neuroprotection. Endocrinology 148, 2936-43 (2007)

185. J. W. Gatson, P. Kaur and M. Singh: Dihydrotestosterone differentially modulates the mitogen-activated protein kinase and the phosphoinositide 3-kinase/Akt pathways through the nuclear and novel membrane androgen receptor in C6 cells. Endocrinology 147, 2028-34 (2006)

186. J. W. Gatson and M. Singh: Activation of a membrane-associated androgen receptor promotes cell death in primary cortical astrocytes. Endocrinology 148, 2458-64 (2007)

187. D. Verthelyi: Sex hormones as immunomodulators in health and disease. Int Immunopharmacol 1, 983-93 (2001)

188. H. H. van den Broek, J. G. Damoiseaux, M. H. De Baets and R. M. Hupperts: The influence of sex hormones on cytokines in multiple sclerosis and experimental autoimmune encephalomyelitis: a review. Mult Scler 11, 349-59 (2005)

189. R. H. Straub: The complex role of estrogens in inflammation. Endocr Rev 28, 521-74 (2007)

190. G. Holdstock, B. F. Chastenay and E. L. Krawitt: Effects of testosterone, oestradiol and progesterone on immune regulation. Clin Exp Immunol 47, 449-56 (1982)

191. A. Bouman, M. J. Heineman and M. M. Faas: Sex hormones and the immune response in humans. Hum Reprod Update 11, 411-23 (2005)

192. K. Asai, N. Hiki, Y. Mimura, T. Ogawa, K. Unou and M. Kaminishi: Gender differences in cytokine secretion by human peripheral blood mononuclear cells: role of estrogen in modulating LPS-induced cytokine secretion in an ex vivo septic model. Shock 16, 340-3 (2001)

193. S. Cenci, G. Toraldo, M. N. Weitzmann, C. Roggia, Y. Gao, W. P. Qian, O. Sierra and R. Pacifici: Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. Proc Natl Acad Sci U S A 100, 10405-10 (2003)

194. A. Ito, A. Matejuk, C. Hopke, H. Drought, J. Dwyer, A. Zamora, S. Subramanian, A. A. Vandenbark and H. Offner: Transfer of severe experimental autoimmune encephalomyelitis by IL-12- and IL-18-potentiated T cells is estrogen sensitive. J Immunol 170, 4802-9 (2003)

195. M. J. Polanczyk, B. D. Carson, S. Subramanian, M. Afentoulis, A. A. Vandenbark, S. F. Ziegler and H. Offner: Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol 173, 2227-30 (2004)

196. J. Reddy, H. Waldner, X. Zhang, Z. Illes, K. W. Wucherpfennig, R. A. Sobel and V. K. Kuchroo: Cutting edge: CD4+CD25+ regulatory T cells contribute to gender differences in susceptibility to experimental autoimmune encephalomyelitis. J Immunol 175, 5591-5 (2005)

197. P. Tai, J. Wang, H. Jin, X. Song, J. Yan, Y. Kang, L. Zhao, X. An, X. Du, X. Chen, S. Wang, G. Xia and B. Wang: Induction of regulatory T cells by physiological level estrogen. J Cell Physiol 214, 456-64 (2008)

198. W. E. Seaman, M. A. Blackman, T. D. Gindhart, J. R. Roubinian, J. M. Loeb and N. Talal: beta-Estradiol reduces natural killer cells in mice. J Immunol 121, 2193-8 (1978)

199. K. C. Dowdell, D. J. Cua, E. Kirkman and S. A. Stohlman: NK cells regulate CD4 responses prior to antigen encounter. J Immunol 171, 234-9 (2003)

200. S. T. Page, S. R. Plymate, W. J. Bremner, A. M. Matsumoto, D. L. Hess, D. W. Lin, J. K. Amory, P. S. Nelson and J. D. Wu: Effect of medical castration on CD4+ CD25+ T cells, CD8+ T cell IFN-gamma expression, and NK cells: a physiological role for testosterone and/or its metabolites. Am J Physiol Endocrinol Metab 290, E856-63 (2006)

201. P. Gourdy, L. M. Araujo, R. Zhu, B. Garmy-Susini, S. Diem, H. Laurell, M. Leite-de-Moraes, M. Dy, J. F. Arnal, F. Bayard and A. Herbelin: Relevance of sexual dimorphism to regulatory T cells: estradiol promotes IFN-gamma production by invariant natural killer T cells. Blood 105, 2415-20 (2005)

202. K. Takahashi, T. Aranami, M. Endoh, S. Miyake and T. Yamamura: The regulatory role of natural killer cells in multiple sclerosis. Brain 127, 1917-27 (2004)

203. M. Araki, T. Kondo, J. E. Gumperz, M. B. Brenner, S. Miyake and T. Yamamura: Th2 bias of CD4+ NKT cells derived from multiple sclerosis in remission. Int Immunol 15, 279-88 (2003)

204. L. T. Mars, J. Novak, R. S. Liblau and A. Lehuen: Therapeutic manipulation of iNKT cells in autoimmunity: modes of action and potential risks. Trends Immunol 25, 471-6 (2004)

205. E. M. Curran, L. J. Berghaus, N. J. Vernetti, A. J. Saporita, D. B. Lubahn and D. M. Estes: Natural killer cells

express estrogen receptor-alpha and estrogen receptor-beta and can respond to estrogen via a non-estrogen receptor-alpha-mediated pathway. Cell Immunol 214, 12-20 (2001)

206. I. F. Hermans, J. D. Silk, U. Gileadi, M. Salio, B. Mathew, G. Ritter, R. Schmidt, A. L. Harris, L. Old and V. Cerundolo: NKT cells enhance CD4+ and CD8+ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. J Immunol 171, 5140-7 (2003)

207. G. Ferlazzo and C. Munz: NK cell compartments and their activation by dendritic cells. J Immunol 172, 1333-9 (2004)

208. X. Liu, K. R. Steffensen, A. Sanna, G. Arru, M. L. Fois, G. Rosati, S. Sotgiu, H. Link, J. A. Gustafsson and Y. M. Huang: Anti-inflammatory nuclear receptor superfamily in multiple sclerosis patients from Sardinia and Sweden. Neurobiol Dis 20, 961-8 (2005)

209. J. Tornwall, A. B. Carey, R. I. Fox and H. S. Fox: Estrogen in autoimmunity: expression of estrogen receptors in thymic and autoimmune T cells. J Gend Specif Med 2, 33-40 (1999)

210. K. L. Phiel, R. A. Henderson, S. J. Adelman and M. M. Elloso: Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. Immunol Lett 97, 107-13 (2005)

211. H. Igarashi, T. Kouro, T. Yokota, P. C. Comp and P. W. Kincade: Age and stage dependency of estrogen receptor expression by lymphocyte precursors. Proc Natl Acad Sci U S A 98, 15131-6 (2001)

212. W. P. Benten, M. Lieberherr, G. Giese, C. Wrehlke, O. Stamm, C. E. Sekeris, H. Mossmann and F. Wunderlich: Functional testosterone receptors in plasma membranes of T cells. Faseb J 13, 123-33 (1999)

213. W. P. Benten, A. Becker, H. P. Schmitt-Wrede and F. Wunderlich: Developmental regulation of intracellular and surface androgen receptors in T cells. Steroids 67, 925-31 (2002)

214. J. Szekeres-Bartho, G. Szekeres, P. Debre, B. Autran and G. Chaouat: Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody. Cell Immunol 125, 273-83 (1990)

215. J. Szekeres-Bartho, B. Polgar, N. Kozma, E. Miko, G. Par, L. Szereday, A. Barakonyi, T. Palkovics, O. Papp and P. Varga: Progesterone-dependent immunomodulation. Chem Immunol Allergy 89, 118-25 (2005)

216. T. A. Tibbetts, F. DeMayo, S. Rich, O. M. Conneely and B. W. O'Malley: Progesterone receptors in the thymus are required for thymic involution during pregnancy and for normal fertility. Proc Natl Acad Sci U S A 96, 12021-6 (1999)

217. N. J. Olsen, G. Olson, S. M. Viselli, X. Gu and W. J. Kovacs: Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. Endocrinology 142, 1278-83 (2001)

218. C. M. Pelfrey, A. C. Cotleur, J. C. Lee and R. A. Rudick: Sex differences in cytokine responses to myelin peptides in multiple sclerosis. J Neuroimmunol 130, 211-23 (2002)

219. L. T. Nguyen, M. Ramanathan, B. Weinstock-Guttman, M. Baier, C. Brownscheidle and L. D. Jacobs: Sex differences in in vitro pro-inflammatory cytokine production from peripheral blood of multiple sclerosis patients. J Neurol Sci 209, 93-9 (2003)

220. C. Lopez, M. Comabella, M. Tintore, J. Sastre-Garriga and X. Montalban: Variations in chemokine receptor and cytokine expression during pregnancy in multiple sclerosis patients. Mult Scler 12, 421-7 (2006)

221. W. Gilmore, L. P. Weiner and J. Correale: Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. J Immunol 158, 446-51 (1997)

222. J. Correale, M. Arias and W. Gilmore: Steroid hormone regulation of cytokine secretion by proteolipid protein-specific CD4+ T cell clones isolated from multiple sclerosis patients and normal control subjects. J Immunol 161, 3365-74 (1998)

223. M. P. Piccinni, M. G. Giudizi, R. Biagiotti, L. Beloni, L. Giannarini, S. Sampognaro, P. Parronchi, R. Manetti, F. Annunziato, C. Livi and et al.: Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 155, 128-33 (1995)

224. S. S. Soldan, A. I. Alvarez Retuerto, N. L. Sicotte and R. R. Voskuhl: Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol 171, 6267-74 (2003)

225. A. Maret, J. D. Coudert, L. Garidou, G. Foucras, P. Gourdy, A. Krust, S. Dupont, P. Chambon, P. Druet, F. Bayard and J. C. Guery: Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor alpha expression in hematopoietic cells. Eur J Immunol 33, 512-21 (2003)

226. W. H. Stimson and I. C. Hunter: Proceedings: An investigation into the immunosuppressive properties of oestrogen. J Endocrinol 69, 42P-43P (1976)

227. A. Noble, A. Giorgini and J. A. Leggat: Cytokine-induced IL-10-secreting CD8 T cells represent a phenotypically distinct suppressor T-cell lineage. Blood 107, 4475-83 (2006)

228. L. Zheng, G. Fisher, R. E. Miller, J. Peschon, D. H. Lynch and M. J. Lenardo: Induction of apoptosis in mature T cells by tumour necrosis factor. Nature 377, 348-51 (1995)

229. M. A. Alexander-Miller, M. A. Derby, A. Sarin, P. A. Henkart and J. A. Berzofsky: Supraoptimal peptide-major histocompatibility complex causes a decrease in bc1-2 levels and allows tumor necrosis factor alpha receptor II-mediated apoptosis of cytotoxic T lymphocytes. J Exp Med 188, 1391-9 (1998)

230. V. P. Badovinac, A. R. Tvinnereim and J. T. Harty: Regulation of antigen-specific CD8+ T cell homeostasis by perforin and interferon-gamma. Science 290, 1354-8 (2000)

231. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group: TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. Neurology 53, 457-65 (1999)

232. L. Steinman: A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 13, 139-45 (2007)

233. S. E. Dunn, S. S. Ousman, R. A. Sobel, L. Zuniga, S. E. Baranzini, S. Youssef, A. Crowell, J. Loh, J. Oksenberg and L. Steinman: Peroxisome proliferator-activated receptor (PPAR)alpha expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. J Exp Med 204, 321-30 (2007)

234. J. Huan, N. Culbertson, L. Spencer, R. Bartholomew, G. G. Burrows, Y. K. Chou, D. Bourdette, S. F. Ziegler, H. Offner and A. A. Vandenbark: Decreased FOXP3 levels in multiple sclerosis patients. J Neurosci Res 81, 45-52 (2005)

235. L. Arruvito, M. Sanz, A. H. Banham and L. Fainboim: Expansion of CD4+CD25+and FOXP3+ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. J Immunol 178, 2572-8 (2007)

236. A. Odyniec, M. Szczepanik, M. P. Mycko, M. Stasiolek, C. S. Raine and K. W. Selmaj: Gammadelta T cells enhance the expression of experimental autoimmune encephalomyelitis by promoting antigen presentation and IL-12 production. J Immunol 173, 682-94 (2004)

237. A. Matejuk, A. C. Bakke, C. Hopke, J. Dwyer, A. A. Vandenbark and H. Offner: Estrogen treatment induces a novel population of regulatory cells, which suppresses experimental autoimmune encephalomyelitis. J Neurosci Res 77, 119-26 (2004)

238. A. Matejuk and M. Afentoulis: Association of CD45(dim)VLA-4(+) cells with the NKT cell lineage and their selective expression of IL-13, IP-15, and CCR3 transcripts. Arch Immunol Ther Exp (Warsz) 54, 183-91 (2006)

239. S. Kim and R. R. Voskuhl: Decreased IL-12 production underlies the decreased ability of male lymph node cells to induce experimental autoimmune encephalomyelitis. J Immunol 162, 5561-8 (1999)

240. S. C. Wilcoxen, E. Kirkman, K. C. Dowdell and S. A. Stohlman: Gender-dependent IL-12 secretion by APC is regulated by IL-10. J Immunol 164, 6237-43 (2000)

241. G. Ferlazzo: Natural killer and dendritic cell liaison: recent insights and open questions. Immunol Lett 101, 12-7 (2005)

242. H. Miyaura and M. Iwata: Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. J Immunol 168, 1087-94 (2002)

243. M. Ryan, L. McCarthy, R. Rappuoli, B. P. Mahon and K. H. Mills: Pertussis toxin potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28. Int Immunol 10, 651-62 (1998)

244. S. M. Kerfoot, E. M. Long, M. J. Hickey, G. Andonegui, B. M. Lapointe, R. C. Zanardo, C. Bonder, W. G. James, S. M. Robbins and P. Kubes: TLR4 contributes to disease-inducing mechanisms resulting in central nervous system autoimmune disease. J Immunol 173, 7070-7 (2004)

245. Z. Y. Wang, D. Yang, Q. Chen, C. A. Leifer, D. M. Segal, S. B. Su, R. R. Caspi, Z. O. Howard and J. J. Oppenheim: Induction of dendritic cell maturation by pertussis toxin and its B subunit differentially initiate Toll-like receptor 4-dependent signal transduction pathways. Exp Hematol 34, 1115-24 (2006)

246. T. Kamradt, P. D. Soloway, D. L. Perkins and M. L. Gefter: Pertussis toxin prevents the induction of peripheral T cell anergy and enhances the T cell response to an encephalitogenic peptide of myelin basic protein. J Immunol 147, 3296-302 (1991)

247. H. H. Hofstetter, C. L. Shive and T. G. Forsthuber: Pertussis toxin modulates the immune response to neuroantigens injected in incomplete Freund's adjuvant: induction of Th1 cells and experimental autoimmune encephalomyelitis in the presence of high frequencies of Th2 cells. J Immunol 169, 117-25 (2002)

248. A. Wakatsuki, P. Borrow, K. Rigley and P. C. Beverley: Cell-surface bound pertussis toxin induces polyclonal T cell responses with high levels of interferon-gamma in the absence of interleukin-12. Eur J Immunol 33, 1859-68 (2003)

249. C. Cassan, E. Piaggio, J. P. Zappulla, L. T. Mars, N. Couturier, F. Bucciarelli, S. Desbois, J. Bauer, D. Gonzalez-Dunia and R. S. Liblau: Pertussis toxin reduces the number of splenic Foxp3+ regulatory T cells. J Immunol 177, 1552-60 (2006)

250. X. Chen, O. M. Howard and J. J. Oppenheim: Pertussis toxin by inducing IL-6 promotes the generation of IL-17-producing CD4 cells. J Immunol 178, 6123-9 (2007)

251. E. P. Blankenhorn, R. J. Butterfield, R. Rigby, L. Cort, D. Giambrone, P. McDermott, K. McEntee, N. Solowski, N. D. Meeker, J. F. Zachary, R. W. Doerge and C. Teuscher: Genetic analysis of the influence of pertussis toxin on experimental allergic encephalomyelitis susceptibility: an environmental agent can override genetic checkpoints. J Immunol 164, 3420-5 (2000)

252. K. L. Medina, G. Smithson and P. W. Kincade: Suppression of B lymphopoiesis during normal pregnancy. J Exp Med 178, 1507-15 (1993)

253. M. del Pilar Martin and N. L. Monson: Potential role of humoral immunity in the pathogenesis of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). Front Biosci 12, 2735-49 (2007)

254. R. Okuyama, T. Abo, S. Seki, T. Ohteki, K. Sugiura, A. Kusumi and K. Kumagai: Estrogen administration activates extrathymic T cell differentiation in the liver. J Exp Med 175, 661-9 (1992)

255. J. E. Staples, T. A. Gasiewicz, N. C. Fiore, D. B. Lubahn, K. S. Korach and A. E. Silverstone: Estrogen receptor alpha is necessary in thymic development and estradiol-induced thymic alterations. J Immunol 163, 4168-74 (1999)

256. A. L. Zoller and G. J. Kersh: Estrogen induces thymic atrophy by eliminating early thymic progenitors and inhibiting proliferation of beta-selected thymocytes. J Immunol 176, 7371-8 (2006)

257. T. Masuzawa, C. Miyaura, Y. Onoe, K. Kusano, H. Ohta, S. Nozawa and T. Suda: Estrogen deficiency stimulates B lymphopoiesis in mouse bone marrow. J Clin Invest 94, 1090-7 (1994)

258. T. M. Ellis, M. T. Moser, P. T. Le, R. C. Flanigan and E. D. Kwon: Alterations in peripheral B cells and B cell progenitors following androgen ablation in mice. Int Immunol 13, 553-8 (2001)

259. W. P. Benten, C. Stephan and F. Wunderlich: B cells express intracellular but not surface receptors for testosterone and estradiol. Steroids 67, 647-54 (2002)

260. T. Paavonen, L. C. Andersson and H. Adlercreutz: Sex hormone regulation of in vitro immune response. Estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures. J Exp Med 154, 1935-45 (1981)

261. F. Rousset, E. Garcia and J. Banchereau: Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. J Exp Med 173, 705-10 (1991)

262. F. Rousset, E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D. H. Hsu, R. Kastelein, K. W. Moore and J. Banchereau: Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci U S A 89, 1890-3 (1992)

263. E. Clerici, E. Bergamasco, E. Ferrario and M. L. Villa: Influence of sex steroids on the antigen-specific primary antibody response in vitro. J Clin Lab Immunol, 34, 71-8 (1991)

264. F. X. Lu, K. Abel, Z. Ma, T. Rourke, D. Lu, J. Torten, M. McChesney and C. J. Miller: The strength of B cell immunity in female rhesus macaques is controlled by CD8+ T cells under the influence of ovarian steroid hormones. Clin Exp Immunol 128, 10-20 (2002)

265. K. L. Medina, A. Strasser and P. W. Kincade: Estrogen influences the differentiation, proliferation, and survival of early B-lineage precursors. Blood 95, 2059-67 (2000)

266. T. S. Thurmond, F. G. Murante, J. E. Staples, A. E. Silverstone, K. S. Korach and T. A. Gasiewicz: Role of estrogen receptor alpha in hematopoietic stem cell development and B lymphocyte maturation in the male mouse. Endocrinology 141, 2309-18 (2000)

267. G. Smithson, J. F. Couse, D. B. Lubahn, K. S. Korach and P. W. Kincade: The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. J Immunol 161, 27-34 (1998)

268. M. S. Bynoe, C. M. Grimaldi and B. Diamond: Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. Proc Natl Acad Sci U S A 97, 2703-8 (2000)

269. C. M. Grimaldi, J. Cleary, A. S. Dagtas, D. Moussai and B. Diamond: Estrogen alters thresholds for B cell apoptosis and activation. J Clin Invest 109, 1625-33 (2002)

270. E. Peeva and M. Zouali: Spotlight on the role of hormonal factors in the emergence of autoreactive B-lymphocytes. Immunol Lett 101, 123-43 (2005)

271. C. P. Genain, B. Cannella, S. L. Hauser and C. S. Raine: Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 5, 170-5 (1999)

272. D. Zhou, R. Srivastava, S. Nessler, V. Grummel, N. Sommer, W. Bruck, H. P. Hartung, C. Stadelmann and B. Hemmer: Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis. Proc Natl Acad Sci U S A 103, 19057-62 (2006)

273. E. K. Mathey, T. Derfuss, M. K. Storch, K. R. Williams, K. Hales, D. R. Woolley, A. Al-Hayani, S. N. Davies, M. N. Rasband, T. Olsson, A. Moldenhauer, S. Velhin, R. Hohlfeld, E. Meinl and C. Linington:

Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 204, 2363-72 (2007)

274. E. Bettelli, D. Baeten, A. Jager, R. A. Sobel and V. K. Kuchroo: Myelin oligodendrocyte glycoprotein-specific T and B cells cooperate to induce a Devic-like disease in mice. J Clin Invest 116, 2393-402 (2006).

275. R. Magliozzi, O. Howell, A. Vora, B. Serafini, R. Nicholas, M. Puopolo, R. Reynolds and F. Aloisi: Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 130, 1089-104 (2007)

276. S. Fillatreau, C. H. Sweenie, M. J. McGeachy, D. Gray and S. M. Anderton: B cells regulate autoimmunity by provision of IL-10. Nat Immunol 3, 944-50 (2002)

277. X. Chen and P. E. Jensen: Cutting edge: primary B lymphocytes preferentially expand allogeneic FoxP3+ CD4 T cells. J Immunol 179, 2046-50 (2007)

278. M. K. Mann, K. Maresz, L. P. Shriver, Y. Tan and B. N. Dittel: B cell regulation of CD4+CD25+ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis. J Immunol 178, 3447-56 (2007)

279. E. Mitsuzawa and T. Yasuda: Experimental allergic encephalitis (EAE) in mice: histological studies on EAE induced by myelin basic protein, and role of pertussis vaccine. Jpn J Exp Med 46, 205-12 (1976)

280. V. H. Secor, W. E. Secor, C. A. Gutekunst and M. A. Brown: Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. J Exp Med 191, 813-22 (2000)

281. J. P. Zappulla, M. Arock, L. T. Mars and R. S. Liblau: Mast cells: new targets for multiple sclerosis therapy? J Neuroimmunol 131, 5-20 (2002)

282. G. D. Gregory, M. Robbie-Ryan, V. H. Secor, J. J. Sabatino, Jr. and M. A. Brown: Mast cells are required for optimal autoreactive T cell responses in a murine model of multiple sclerosis. Eur J Immunol 35, 3478-86 (2005)

283. S. Musio, B. Gallo, S. Scabeni, M. Lapilla, P. L. Poliani, G. Matarese, H. Ohtsu, S. J. Galli, R. Mantegazza, L. Steinman and R. Pedotti: A key regulatory role for histamine in experimental autoimmune encephalomyelitis: disease exacerbation in histidine decarboxylase-deficient mice. J Immunol 176, 17-26 (2006)

284. R. Pedotti, J. J. De Voss, L. Steinman and S. J. Galli: Involvement of both 'allergic' and 'autoimmune' mechanisms in EAE, MS and other autoimmune diseases. Trends Immunol 24, 479-84 (2003)

285. M. Jutel, T. Watanabe, S. Klunker, M. Akdis, O. A. Thomet, J. Malolepszy, T. Zak-Nejmark, R. Koga, T. Kobayashi, K. Blaser and C. A. Akdis: Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 413, 420-5 (2001)

286. C. Teuscher, M. E. Poynter, H. Offner, A. Zamora, T. Watanabe, P. D. Fillmore, J. F. Zachary and E. P. Blankenhorn: Attenuation of Th1 effector cell responses and susceptibility to experimental allergic encephalomyelitis in histamine H2 receptor knockout mice is due to dysregulation of cytokine production by antigen-presenting cells. Am J Pathol 164, 883-92 (2004)

287. C. Teuscher, M. Subramanian, R. Noubade, J. F. Gao, H. Offner, J. F. Zachary and E. P. Blankenhorn: Central histamine H3 receptor signaling negatively regulates susceptibility to autoimmune inflammatory disease of the CNS. Proc Natl Acad Sci U S A 104, 10146-51 (2007)

288. T. C. Theoharides, V. Dimitriadou, R. Letourneau, J. J. Rozniecki, H. Vliagoftis and W. Boucher: Synergistic action of estradiol and myelin basic protein on mast cell secretion and brain myelin changes resembling early stages of demyelination. Neuroscience 57, 861-71 (1993)

289. L. Zhao, T. W. Wu and R. D. Brinton: Estrogen receptor subtypes alpha and beta contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. Brain Res 1010, 22-34 (2004)

290. M. Zaitsu, S. Narita, K. C. Lambert, J. J. Grady, D. M. Estes, E. M. Curran, E. G. Brooks, C. S. Watson, R. M. Goldblum and T. Midoro-Horiuti: Estradiol activates mast cells via a non-genomic estrogen receptor-alpha and calcium influx. Mol Immunol 44, 1977-85 (2007)

291. M. S. Kim, H. J. Chae, T. Y. Shin, H. M. Kim and H. R. Kim: Estrogen regulates cytokine release in human mast cells. Immunopharmacol Immunotoxicol 23, 495-504 (2001)

292. M. Vasiadi, D. Kempuraj, W. Boucher, D. Kalogeromitros and T. C. Theoharides: Progesterone inhibits mast cell secretion. Int J Immunopathol Pharmacol 19, 787-94 (2006)

293. M. P. Belot, L. Abdennebi-Najar, F. Gaudin, M. Lieberherr, V. Godot, J. Taieb, D. Emilie and V. Machelon: Progesterone reduces the migration of mast cells toward the chemokine stromal cell-derived factor-1/CXCL12 with an accompanying decrease in CXCR4 receptors. Am J Physiol Endocrinol Metab 292, E1410-7 (2007)

294. J. J. Hendriks, C. E. Teunissen, H. E. de Vries and C. D. Dijkstra: Macrophages and neurodegeneration. Brain Res Brain Res Rev 48, 185-95 (2005)

295. C. Jack, F. Ruffini, A. Bar-Or and J. P. Antel: Microglia and multiple sclerosis. J Neurosci Res 81, 363-73 (2005)

296. D. J. Cua and S. A. Stohlman: In vivo effects of T helper cell type 2 cytokines on macrophage antigen-

presenting cell induction of T helper subsets. J Immunol 159, 5834-40 (1997)

297. T. C. Chao, P. J. Van Alten, J. A. Greager and R. J. Walter: Steroid sex hormones regulate the release of tumor necrosis factor by macrophages. Cell Immunol 160, 43-9 (1995)

298. R. Deshpande, H. Khalili, R. G. Pergolizzi, S. D. Michael and M. D. Chang: Estradiol down-regulates LPS-induced cytokine production and NFkB activation in murine macrophages. Am J Reprod Immunol 38, 46-54 (1997)

299. E. Vegeto, S. Ghisletti, C. Meda, S. Etteri, S. Belcredito and A. Maggi: Regulation of the lipopolysaccharide signal transduction pathway by 17beta-estradiol in macrophage cells. J Steroid Biochem Mol Biol 91, 59-66 (2004)

300. S. Ghisletti, C. Meda, A. Maggi and E. Vegeto: 17beta-estradiol inhibits inflammatory gene expression by controlling NF-kappaB intracellular localization. Mol Cell Biol 25, 2957-68 (2005)

301. T. Suzuki, T. Shimizu, H. P. Yu, Y. C. Hsieh, M. A. Choudhry, K. I. Bland and I. H. Chaudry: Estrogen receptor-alpha predominantly mediates the salutary effects of 17beta-estradiol on splenic macrophages following trauma-hemorrhage. Am J Physiol Cell Physiol 293, C978-84 (2007)

302. K. C. Lambert, E. M. Curran, B. M. Judy, G. N. Milligan, D. B. Lubahn and D. M. Estes: Estrogen receptor alpha (ERalpha) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17beta-estradiol acts through ERalpha to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. J Immunol 175, 5716-23 (2005)

303. J. A. McCrohon, A. K. Death, S. Nakhla, W. Jessup, D. J. Handelsman, K. K. Stanley and D. S. Celermajer: Androgen receptor expression is greater in macrophages from male than from female donors. A sex difference with implications for atherogenesis. Circulation 101, 224-6 (2000)

304. K. Ahmadi and A. B. McCruden: Androgen receptor in macrophages of male rat is greater than in female. Am J Immunol 1, 48-54 (2005)

305. Z. Guo, J. Krucken, W. P. Benten and F. Wunderlich: Estradiol-induced nongenomic calcium signaling regulates genotropic signaling in macrophages. J Biol Chem 277, 7044-50 (2002)

306. W. P. Benten, Z. Guo, J. Krucken and F. Wunderlich: Rapid effects of androgens in macrophages. Steroids 69, 585-90 (2004)

307. M. Ding, J. L. Wong, N. E. Rogers, L. J. Ignarro and R. R. Voskuhl: Gender differences of inducible nitric oxide production in SJL/J mice with experimental autoimmune encephalomyelitis. J Neuroimmunol 77, 99-106 (1997)

308. D. O. Willenborg, M. A. Staykova and W. B. Cowden: Our shifting understanding of the role of nitric oxide in autoimmune encephalomyelitis: a review. J Neuroimmunol 100, 21-35 (1999)

309. A. E. Juedes and N. H. Ruddle: Resident and infiltrating central nervous system APCs regulate the emergence and resolution of experimental autoimmune encephalomyelitis. J Immunol 166, 5168-75 (2001)

310. S. L. Manuel, S. Rahman, B. Wigdahl, Z. K. Khan and P. Jain: Dendritic cells in autoimmune diseases and neuroinflammatory disorders. Front Biosci 12, 4315-35 (2007)

311. H. Y. Liu, A. C. Buenafe, A. Matejuk, A. Ito, A. Zamora, J. Dwyer, A. A. Vandenbark and H. Offner: Estrogen inhibition of EAE involves effects on dendritic cell function. J Neurosci Res 70, 238-48 (2002)

312. Q. H. Zhang, Y. Z. Hu, J. Cao, Y. Q. Zhong, Y. F. Zhao and Q. B. Mei: Estrogen influences the differentiation, maturation and function of dendritic cells in rats with experimental autoimmune encephalomyelitis. Acta Pharmacol Sin 25, 508-13 (2004)

313. A. Pettersson, C. Ciumas, V. Chirsky, H. Link, Y. M. Huang and B. G. Xiao: Dendritic cells exposed to estrogen in vitro exhibit therapeutic effects in ongoing experimental allergic encephalomyelitis. J Neuroimmunol 156, 58-65 (2004)

314. V. Paharkova-Vatchkova, R. Maldonado and S. Kovats: Estrogen preferentially promotes the differentiation of CD11c+ CD11b (intermediate) dendritic cells from bone marrow precursors. J Immunol 172, 1426-36 (2004)

315. V. Douin-Echinard, S. Laffont, C. Seillet, L. Delpy, A. Krust, P. Chambon, P. Gourdy, J. F. Arnal and J. C. Guery: Estrogen receptor alpha, but not beta, is required for optimal dendritic cell differentiation and CD40-induced cytokine production. J Immunol 180, 3661-9 (2008)

316. C. L. Butts, S. A. Shukair, K. M. Duncan, E. Bowers, C. Horn, E. Belyavskaya, L. Tonelli and E. M. Sternberg: Progesterone inhibits mature rat dendritic cells in a receptor-mediated fashion. Int Immunol 19, 287-96 (2007)

317. B. Huck, T. Steck, M. Habersack, J. Dietl and U. Kammerer: Pregnancy associated hormones modulate the cytokine production but not the phenotype of PBMC-derived human dendritic cells. Eur J Obstet Gynecol Reprod Biol 122, 85-94 (2005)

318. D. N. Krause, S. P. Duckles and D. A. Pelligrino: Influence of sex steroid hormones on cerebrovascular function. J Appl Physiol 101, 1252-61 (2006)

319. J. F. Arnal, V. Douin-Echinard, F. Tremollieres, A. D. Terrisse, P. Sie, B. Payrastre, J. C. Guery, F. Bayard and P. Gourdy: Understanding the controversy about hormonal replacement therapy: insights from estrogen effects on experimental and clinical atherosclerosis. Arch Mal Coeur Vaiss, 100, 554-62 (2007)

320. J. B. Dietrich: Endothelial cells of the blood-brain barrier: a target for glucocorticoids and estrogens? Front Biosci 9, 684-93 (2004)

321. M. C. Cid, H. K. Kleinman, D. S. Grant, H. W. Schnaper, A. S. Fauci and G. S. Hoffman: Estradiol enhances leukocyte binding to tumor necrosis factor (TNF)-stimulated endothelial cells via an increase in TNF-induced adhesion molecules E-selectin, intercellular adhesion molecule type 1, and vascular cell adhesion molecule type 1. J Clin Invest 93, 17-25 (1994)

322. T. Caulin-Glaser, C. A. Watson, R. Pardi and J. R. Bender: Effects of 17beta-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest 98, 36-42 (1996)

323. M. Otsuki, H. Saito, X. Xu, S. Sumitani, H. Kouhara, T. Kishimoto and S. Kasayama: Progesterone, but not medroxyprogesterone, inhibits vascular cell adhesion molecule-1 expression in human vascular endothelial cells. Arterioscler Thromb Vasc Biol 21, 243-8 (2001)

324. T. K. Mukherjee, H. Dinh, G. Chaudhuri and L. Nathan: Testosterone attenuates expression of vascular cell adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. Proc Natl Acad Sci U S A 99, 4055-60 (2002)

325. T. K. Mukherjee, L. Nathan, H. Dinh, S. T. Reddy and G. Chaudhuri: 17-epiestriol, an estrogen metabolite, is more potent than estradiol in inhibiting vascular cell adhesion molecule 1 (VCAM-1) mRNA expression. J Biol Chem 278, 11746-52 (2003)

326. D. Xing, A. Miller, L. Novak, R. Rocha, Y. F. Chen and S. Oparil: Estradiol and progestins differentially modulate leukocyte infiltration after vascular injury. Circulation 109, 234-41 (2004)

327. F. Sohrabji and D. K. Lewis: Estrogen-BDNF interactions: implications for neurodegenerative diseases. Front Neuroendocrinol 27, 404-14 (2006)

328. O. Z. Chi, S. Barsoum, Y. Wen, X. Liu and H. R. Weiss: 17beta-estradiol prevents blood-brain barrier disruption induced by VEGF. Horm Metab Res 36, 272-6 (2004)

329. H. S. Kang, H. S. Ahn, H. J. Kang and M. C. Gye: Effect of estrogen on the expression of occludin in ovariectomized mouse brain. Neurosci Lett 402, 30-4 (2006)

330. R. Liu, Y. Wen, E. Perez, X. Wang, A. L. Day, J. W. Simpkins and S. H. Yang: 17beta-Estradiol attenuates blood-brain barrier disruption induced by cerebral ischemia-reperfusion injury in female rats. Brain Res 1060, 55-61 (2005)

331. M. E. O'Donnell, T. I. Lam, L. Q. Tran, S. Foroutan and S. E. Anderson: Estradiol reduces activity of the blood-brain barrier Na-K-Cl cotransporter and decreases edema formation in permanent middle cerebral artery occlusion. J Cereb Blood Flow Metab 26, 1234-49 (2006)

332. S. Santagati, R. C. Melcangi, F. Celotti, L. Martini and A. Maggi: Estrogen receptor is expressed in different types of glial cells in culture. J Neurochem 63, 2058-64 (1994)

333. B. S. McEwen and S. E. Alves: Estrogen actions in the central nervous system. Endocr Rev 20, 279-307 (1999)

334. I. Azcoitia, D. Garcia-Ovejero, J. A. Chowen and L. M. Garcia-Segura: Astroglia play a key role in the neuroprotective actions of estrogen. Prog Brain Res 132, 469-78 (2001)

335. D. Garcia-Ovejero, S. Veiga, L. M. Garcia-Segura and L. L. Doncarlos: Glial expression of estrogen and androgen receptors after rat brain injury. J Comp Neurol 450, 256-71 (2002)

336. J. Pawlak, M. Karolczak, A. Krust, P. Chambon and C. Beyer: Estrogen receptor-alpha is associated with the plasma membrane of astrocytes and coupled to the MAP/Src-kinase pathway. Glia 50, 270-5 (2005)

337. F. O. Dimayuga, J. L. Reed, G. A. Carnero, C. Wang, E. R. Dimayuga, V. M. Dimayuga, A. Perger, M. E. Wilson, J. N. Keller and A. J. Bruce-Keller: Estrogen and brain inflammation: effects on microglial expression of MHC, costimulatory molecules and cytokines. J Neuroimmunol 161, 123-36 (2005)

338. X. Wang and Y. Suzuki: Microglia produce IFN-gamma independently from T cells during acute toxoplasmosis in the brain. J Interferon Cytokine Res 27, 599-605 (2007)

339. K. M. Dhandapani, F. M. Wade, V. B. Mahesh and D. W. Brann: Astrocyte-derived transforming growth factor-{beta} mediates the neuroprotective effects of 17{beta}-estradiol: involvement of nonclassical genomic signaling pathways. Endocrinology 146, 2749-59 (2005)

340. J. Pawlak, V. Brito, E. Kuppers and C. Beyer: Regulation of glutamate transporter GLAST and GLT-1 expression in astrocytes by estrogen. Brain Res Mol Brain Res 138, 1-7 (2005)

341. J. Garcia-Estrada, J. A. Del Rio, S. Luquin, E. Soriano and L. M. Garcia-Segura: Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. Brain Res 628, 271-8 (1993)

342. G. Barreto, S. Veiga, I. Azcoitia, L. M. Garcia-Segura and D. Garcia-Ovejero: Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. Eur J Neurosci 25, 3039-46 (2007)

343. A. J. Bruce-Keller, J. L. Keeling, J. N. Keller, F. F. Huang, S. Camondola and M. P. Mattson: Antiinflammatory effects of estrogen on microglial activation. Endocrinology 141, 3646-56 (2000)

344. E. Vegeto, G. Pollio, P. Ciana and A. Maggi: Estrogen blocks inducible nitric oxide synthase accumulation in LPS-activated microglia cells. Exp Gerontol 35, 1309-16 (2000)

345. E. Vegeto, S. Belcredito, S. Etteri, S. Ghisletti, A. Brusadelli, C. Meda, A. Krust, S. Dupont, P. Ciana, P. Chambon and A. Maggi: Estrogen receptor-alpha mediates the brain antiinflammatory activity of estradiol. Proc Natl Acad Sci U S A 100, 9614-9 (2003)

346. E. Vegeto, S. Belcredito, S. Ghisletti, C. Meda, S. Etteri and A. Maggi: The endogenous estrogen status regulates microglia reactivity in animal models of neuroinflammation. Endocrinology 147, 2263-72 (2006)

347. E. Vegeto, C. Bonincontro, G. Pollio, A. Sala, S. Viappiani, F. Nardi, A. Brusadelli, B. Viviani, P. Ciana and A. Maggi: Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. J Neurosci 21, 1809-18 (2001)

348. A. E. Baker, V. M. Brautigam and J. J. Watters: Estrogen modulates microglial inflammatory mediator production via interactions with estrogen receptor beta. Endocrinology 145, 5021-32 (2004)

349. X. Liu, X. L. Fan, Y. Zhao, G. R. Luo, X. P. Li, R. Li and W. D. Le: Estrogen provides neuroprotection against activated microglia-induced dopaminergic neuronal injury through both estrogen receptor-alpha and estrogen receptor-beta in microglia. J Neurosci Res 81, 653-65 (2005)

350. S. K. Finley and M. F. Kritzer: Immunoreactivity for intracellular androgen receptors in identified subpopulations of neurons, astrocytes and oligodendrocytes in primate prefrontal cortex. J Neurobiol 40, 446-57 (1999)

351. B. Lorenz, L. M. Garcia-Segura and L. L. DonCarlos: Cellular phenotype of androgen receptor-immunoreactive nuclei in the developing and adult rat brain. J Comp Neurol 492, 456-68 (2005)

352. L. L. DonCarlos, S. Sarkey, B. Lorenz, I. Azcoitia, D. Garcia-Ovejero, C. Huppenbauer and L. M. Garcia-Segura: Novel cellular phenotypes and subcellular sites for androgen action in the forebrain. Neuroscience 138, 801-7 (2006)

353. J. C. Turcotte and J. D. Blaustein: Immunocytochemical localization of midbrain estrogen receptor- and progestin receptor-containing cells in female guinea pigs. J Comp Neurol, 328, 76-87 (1993)

354. S. E. Alves, N. G. Weiland, S. Hayashi and B. S. McEwen: Immunocytochemical localization of nuclear estrogen receptors and progestin receptors within the rat dorsal raphe nucleus. J Comp Neurol 391, 322-34 (1998)

355. R. E. Scott, X. S. Wu-Peng and D. W. Pfaff: Regulation and expression of progesterone receptor mRNA isoforms A and B in the male and female rat hypothalamus and pituitary following oestrogen treatment. J Neuroendocrinol 14, 175-83 (2002)

356. M. Fodor, F. W. van Leeuwen and D. F. Swaab: Differences in postmortem stability of sex steroid receptor immunoreactivity in rat brain. J Histochem Cytochem 50, 641-50 (2002)

357. F. Labombarda, S. L. Gonzalez, M. C. Deniselle, G. P. Vinson, M. Schumacher, A. F. De Nicola and R. Guennoun: Effects of injury and progesterone treatment on progesterone receptor and progesterone binding protein 25-Dx expression in the rat spinal cord. J Neurochem 87, 902-13 (2003)

358. I. Jung-Testas, M. Renoir, H. Bugnard, G. L. Greene and E. E. Baulieu: Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. J Steroid Biochem Mol Biol 41, 621-31 (1992)

359. N. J. MacLusky and B. S. McEwen: Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. Nature 274, 276-8 (1978)

360. K. Lieb, S. Engels and B. L. Fiebich: Inhibition of LPS-induced iNOS and NO synthesis in primary rat microglial cells. Neurochem Int 42, 131-7 (2003)

361. T. Coughlan, C. Gibson and S. Murphy: Modulatory effects of progesterone on inducible nitric oxide synthase expression in vivo and in vitro. J Neurochem 93, 932-42 (2005)

362. M. Djebaili, Q. Guo, E. H. Pettus, S. W. Hoffman and D. G. Stein: The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. J Neurotrauma 22, 106-18 (2005)

363. J. He, C. O. Evans, S. W. Hoffman, N. M. Oyesiku and D. G. Stein: Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. Exp Neurol 189, 404-12 (2004)

364. K. Sinchak, R. H. Mills, L. Tao, P. LaPolt, J. K. Lu and P. Micevych: Estrogen induces de novo progesterone synthesis in astrocytes. Dev Neurosci 25, 343-8 (2003)

365. T. Takao, N. Flint, L. Lee, X. Ying, J. Merrill and K. J. Chandross: 17beta-estradiol protects oligodendrocytes

from cytotoxicity induced cell death. J Neurochem 89, 660-73 (2004)

366. D. N. Arvanitis, H. Wang, R. D. Bagshaw, J. W. Callahan and J. M. Boggs: Membrane-associated estrogen receptor and caveolin-1 are present in central nervous system myelin and oligodendrocyte plasma membranes. J Neurosci Res 75, 603-13 (2004)

367. J. J. Curry, 3rd and L. M. Heim: Brain myelination after neonatal administration of oestradiol. Nature 209, 915-6 (1966)

368. A. Caruso, V. Di Giorgi Gerevini, M. Castiglione, F. Marinelli, V. Tomassini, C. Pozzilli, A. Caricasole, V. Bruno, F. Caciagli, A. Moretti, F. Nicoletti and D. Melchiorri: Testosterone amplifies excitotoxic damage of cultured oligodendrocytes. J Neurochem 88, 1179-85 (2004)

369. R. B. Simerly, C. Chang, M. Muramatsu and L. W. Swanson: Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 294, 76-95 (1990)

370. D. W. Pfaff, J. L. Gerlach, B. S. McEwen, M. Ferin, P. Carmel and E. A. Zimmerman: Autoradiographic localization of hormone-concentrating cells in the brain of the female rhesus monkey. J Comp Neurol 170, 279-93 (1976)

immunoreactivity in rat forebrain. Neuroendocrinology 66, 63-7 (1997)

372. P. J. Shughrue, M. V. Lane and I. Merchenthaler: Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J Comp Neurol 388, 507-25 (1997)

373. M. Osterlund, G. G. Kuiper, J. A. Gustafsson and Y. L. Hurd: Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. Brain Res Mol Brain Res 54, 175-80 (1998)

374. S. Lumbroso, F. Sandillon, V. Georget, J. M. Lobaccaro, A. O. Brinkmann, A. Privat and C. Sultan: Immunohistochemical localization and immunoblotting of androgen receptor in spinal neurons of male and female rats. Eur J Endocrinol 134, 626-32 (1996)

375. M. K. Osterlund, K. Grandien, E. Keller and Y. L. Hurd: The human brain has distinct regional expression patterns of estrogen receptor alpha mRNA isoforms derived from alternative promoters. J Neurochem 75, 1390-7 (2000)

376. L. L. DonCarlos, D. Garcia-Ovejero, S. Sarkey, L. M. Garcia-Segura and I. Azcoitia: Androgen receptor immunoreactivity in forebrain axons and dendrites in the rat. Endocrinology 144, 3632-8 (2003)

377. Y. Feng and P. Gregor: Cloning of a novel member of the G protein-coupled receptor family related to peptide receptors. Biochem Biophys Res Commun 231, 651-4 (1997)

378. B. F. O'Dowd, T. Nguyen, A. Marchese, R. Cheng, K. R. Lynch, H. H. Heng, L. F. Kolakowski, Jr. and S. R. George: Discovery of three novel G-protein-coupled receptor genes. Genomics 47, 310-3 (1998)

379. T. Funakoshi, A. Yanai, K. Shinoda, M. M. Kawano and Y. Mizukami: G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. Biochem Biophys Res Commun 346, 904-10 (2006)

380. E. Brailoiu, S. L. Dun, G. C. Brailoiu, K. Mizuo, L. A. Sklar, T. I. Oprea, E. R. Prossnitz and N. J. Dun: Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. J Endocrinol 193, 311-21 (2007)

381. H. Sakamoto, K. I. Matsuda, K. Hosokawa, M. Nishi, J. F. Morris, E. R. Prossnitz and M. Kawata: Expression of GPR30, a G protein-Coupled Membrane Estrogen Receptor, in Oxytocin Neurons of the Rat Paraventricular and Supraoptic Nuclei. Endocrinology 148, 5842-50 (2007)

382. L. M. Garcia-Segura, I. Azcoitia and L. L. DonCarlos: Neuroprotection by estradiol. Prog Neurobiol 63, 29-60 (2001)

383. D. N. Bryant, L. C. Sheldahl, L. K. Marriott, R. A. Shapiro and D. M. Dorsa: Multiple pathways transmit neuroprotective effects of gonadal steroids. Endocrine 29, 199-207 (2006)

384. A. Ardeshiri, M. H. Kelley, I. P. Korner, P. D. Hurn and P. S. Herson: Mechanism of progesterone neuroprotection of rat cerebellar Purkinje cells following oxygen-glucose deprivation. Eur J Neurosci 24, 2567-74 (2006)

385. A. Nicot, P. V. Ratnakar, Y. Ron, C. C. Chen and S. Elkabes: Regulation of gene expression in experimental autoimmune encephalomyelitis indicates early neuronal dysfunction. Brain 126, 398-412 (2003)

386. A. Nicot, M. Kurnellas and S. Elkabes: Temporal pattern of plasma membrane calcium ATPase 2 expression in the spinal cord correlates with the course of clinical symptoms in two rodent models of autoimmune encephalomyelitis. Eur J Neurosci 21, 2660-70 (2005)

387. B. Zhu, L. Luo, G. R. Moore, D. W. Paty and M. S. Cynader: Dendritic and synaptic pathology in experimental autoimmune encephalomyelitis. Am J Pathol 162, 1639-50 (2003)

388. R. Dutta, J. McDonough, X. Yin, J. Peterson, A. Chang, T. Torres, T. Gudz, W. B. Macklin, D. A. Lewis, R. J. Fox, R. Rudick, K. Mirnics and B. D. Trapp: Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol 59, 478-89 (2006)

389. D. Yokomaku, T. Numakawa, Y. Numakawa, S. Suzuki, T. Matsumoto, N. Adachi, C. Nishio, T. Taguchi and H. Hatanaka: Estrogen enhances depolarization-induced glutamate release through activation of phosphatidylinositol 3-kinase and mitogen-activated protein kinase in cultured hippocampal neurons. Mol Endocrinol 17, 831-44 (2003)

390. C. A. Singer, K. L. Rogers, T. M. Strickland and D. M. Dorsa: Estrogen protects primary cortical neurons from glutamate toxicity. Neurosci Lett 212, 13-6 (1996)

391. C. A. Singer, X. A. Figueroa-Masot, R. H. Batchelor and D. M. Dorsa: The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. J Neurosci 19, 2455-63 (1999)

392. P. Kaur, P. K. Jodhka, W. A. Underwood, C. A. Bowles, N. C. de Fiebre, C. M. de Fiebre and M. Singh: Progesterone increases brain-derived neuroptrophic factor expression and protects against glutamate toxicity in a mitogen-activated protein kinase- and phosphoinositide-3 kinase-dependent manner in cerebral cortical explants. J Neurosci Res 85, 2441-9 (2007)

393. H. V. Carswell, I. M. Macrae, L. Gallagher, E. Harrop and K. J. Horsburgh: Neuroprotection by a selective estrogen receptor beta agonist in a mouse model of global ischemia. Am J Physiol Heart Circ Physiol 287, H1501-4 (2004)

394. N. R. Miller, T. Jover, H. W. Cohen, R. S. Zukin and A. M. Etgen: Estrogen can act via estrogen receptor alpha and beta to protect hippocampal neurons against global ischemia-induced cell death. Endocrinology 146, 3070-9 (2005)

395. M. D. Gonzalez-Vidal, M. Cervera-Gaviria, R. Ruelas, A. Escobar, G. Morali and M. Cervantes: Progesterone: protective effects on the cat hippocampal neuronal damage due to acute global cerebral ischemia. Arch Med Res 29, 117-24 (1998)

396. Y. Kumon, S. C. Kim, P. Tompkins, A. Stevens, S. Sakaki and C. M. Loftus: Neuroprotective effect of postischemic administration of progesterone in spontaneously hypertensive rats with focal cerebral ischemia. J Neurosurg 92, 848-52 (2000)

397. S. J. Murphy, M. T. Littleton-Kearney and P. D. Hurn: Progesterone administration during reperfusion, but not preischemia alone, reduces injury in ovariectomized rats. J Cereb Blood Flow Metab 22, 1181-8 (2002)

398. M. Bialek, P. Zaremba, K. K. Borowicz and S. J. Czuczwar: Neuroprotective role of testosterone in the nervous system. Pol J Pharmacol 56, 509-18 (2004)

399. R. Orlando, A. Caruso, G. Molinaro, M. Motolese, F. Matrisciano, G. Togna, D. Melchiorri, F. Nicoletti and V. Bruno: Nanomolar concentrations of anabolic-androgenic steroids amplify excitotoxic neuronal death in mixed mouse cortical cultures. Brain Res 1165, 21-9 (2007)

400. S. Hulley, D. Grady, T. Bush, C. Furberg, D. Herrington, B. Riggs and E. Vittinghoff: Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. Jama 280, 605-13 (1998)

401. J. E. Rossouw, G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. L. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard, K. C. Johnson, J. M. Kotchen and J. Ockene: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. Jama 288, 321-33 (2002)

402. V. Beral, D. Bull, J. Green and G. Reeves: Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet 369, 1703-10 (2007)

403. M. A. Espeland, S. R. Rapp, S. A. Shumaker, R. Brunner, J. E. Manson, B. B. Sherwin, J. Hsia, K. L. Margolis, P. E. Hogan, R. Wallace, M. Dailey, R. Freeman and J. Hays: Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. Jama 291, 2959-68 (2004)

404. J. L. Turgeon, M. C. Carr, P. M. Maki, M. E. Mendelsohn and P. M. Wise: Complex actions of sex steroids in adipose tissue, the cardiovascular system, and brain: Insights from basic science and clinical studies. Endocr Rev 27, 575-605 (2006)

405. E. B. Claus, P. M. Black, M. L. Bondy, L. Calvocoressi, J. M. Schildkraut, J. L. Wiemels and M. Wrensch: Exogenous hormone use and meningioma risk: what do we tell our patients? Cancer 110, 471-6 (2007)

406. S. B. Hulley and D. Grady: The WHI estrogen-alone trial--do things look any better? Jama 291, 1769-71 (2004)

407. T. N. Thomas, J. A. Rhodin, L. Clark, A. Garces and M. Bryant: A comparison of the anti-inflammatory activities of conjugated estrogens and 17-beta estradiol. Inflamm Res 52, 452-60 (2003)

408. J. M. Daniel, J. L. Hulst and J. L. Berbling: Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology 147, 607-14 (2006)

409. S. Suzuki, C. M. Brown, C. D. Dela Cruz, E. Yang, D. A. Bridwell and P. M. Wise: Timing of estrogen therapy after ovariectomy dictates the efficacy of its neuroprotective and antiinflammatory actions. Proc Natl Acad Sci U S A 104, 6013-8 (2007)

410. J. E. Rossouw, R. L. Prentice, J. E. Manson, L. Wu, D. Barad, V. M. Barnabei, M. Ko, A. Z. LaCroix, K. L.

Margolis and M. L. Stefanick: Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. Jama 297, 1465-77 (2007)

411. M. R. Vickers, J. Martin and T. W. Meade: The Women's international study of long-duration oestrogen after menopause (WISDOM): a randomised controlled trial. BMC Womens Health 7, 2 (2007)

412. E. Barrett-Connor and C. A. Stuenkel: Hormone replacement therapy (HRT)--risks and benefits. Int J Epidemiol 30, 423-6 (2001)

413. P. P. Zandi, M. C. Carlson, B. L. Plassman, K. A. Welsh-Bohmer, L. S. Mayer, D. C. Steffens and J. C. Breitner: Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. Jama 288, 2123-9 (2002)

414. S. M. Harman, E. A. Brinton, M. Cedars, R. Lobo, J. E. Manson, G. R. Merriam, V. M. Miller, F. Naftolin and N. Santoro: KEEPS: The Kronos Early Estrogen Prevention Study. Climacteric 8, 3-12 (2005)

415. S. Sasson and A. C. Notides: Estriol and estrone interaction with the estrogen receptor. II. Estriol and estrone-induced inhibition of the cooperative binding of (3H)estradiol to the estrogen receptor. J Biol Chem 258, 8118-22 (1983)

416. J. H. Clark and B. M. Markaverich: The agonistic and antagonistic actions of estriol. J Steroid Biochem 20, 1005-13 (1984)

417. R. E. Muller, A. M. Traish and H. H. Wotiz: Interaction of estradiol and estriol with uterine estrogen receptor in vivo and in excised uteri or cell suspensions at 37 C: noncooperative estradiol binding and absence of estriol inhibition of estradiol-induced receptor activation and transformation. Endocrinology 117, 1839-47 (1985)

418. M. Melamed, E. Castano, A. C. Notides and S. Sasson: Molecular and kinetic basis for the mixed agonist/antagonist activity of estriol. Mol Endocrinol 11, 1868-78 (1997)

419. E. Weiderpass, J. A. Baron, H. O. Adami, C. Magnusson, A. Lindgren, R. Bergstrom, N. Correia and I. Persson: Low-potency oestrogen and risk of endometrial cancer: a case-control study. Lancet 353, 1824-8 (1999)

420. C. M. Bamberger, T. Else, A. M. Bamberger, F. U. Beil and H. M. Schulte: Dissociative glucocorticoid activity of medroxyprogesterone acetate in normal human lymphocytes. J Clin Endocrinol Metab 84, 4055-61 (1999)

421. K. Miyagawa, J. Rosch, F. Stanczyk and K. Hermsmeyer: Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. Nat Med 3, 324-7 (1997)

422. J. Nilsen and R. D. Brinton: Impact of progestins on estrogen-induced neuroprotection: synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. Endocrinology 143, 205-12 (2002)

423. J. Nilsen and R. D. Brinton: Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. Proc Natl Acad Sci U S A 100, 10506-11 (2003)

424. J. Nilsen, A. Morales and R. D. Brinton: Medroxyprogesterone acetate exacerbates glutamate excitotoxicity. Gynecol Endocrinol 22, 355-61 (2006)

425. T. A. Grese and J. A. Dodge: Selective estrogen receptor modulators (SERMs). Curr Pharm Des 4, 71-92 (1998)

426. H. Offner, A. Zamora, H. Drought, A. Matejuk, D. L. Auci, E. E. Morgan, A. A. Vandenbark and C. L. Reading: A synthetic androstene derivative and a natural androstene metabolite inhibit relapsing-remitting EAE. J Neuroimmunol 130, 128-39 (2002)

427. C. C. Chadwick, S. Chippari, E. Matelan, L. Borges-Marcucci, A. M. Eckert, J. C. Keith, Jr., L. M. Albert, Y. Leathurby, H. A. Harris, R. A. Bhat, M. Ashwell, E. Trybulski, R. C. Winneker, S. J. Adelman, R. J. Steffan and D. C. Harnish: Identification of pathway-selective estrogen receptor ligands that inhibit NF-kappaB transcriptional activity. Proc Natl Acad Sci U S A 102, 2543-8 (2005)

428. D. R. Maccio, Y. Ditamo, A. L. Degano and G. A. Roth: Interaction between gonadal steroids and neuroimmune system in acute experimental autoimmune encephalomyelitis (EAE) in Wistar rats. Autoimmunity 37, 17-25 (2004)

429. Y. Okuda, M. Okuda and C. C. Bernard: Gender does not influence the susceptibility of C57BL/6 mice to develop chronic experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein. Immunol Lett 81, 25-9 (2002)

430. R. J. Liedtke, J. P. Greaves, Jr., J. D. Batjer and B. Busby: 125I-radioimmunoassay for unconjugated estriol in serum of pregnant women. Clin Chem 24, 1100-4 (1978)

431. C. Longcope and J. H. Pratt: Relationship between urine and plasma estrogen ratios. Cancer Res 38, 4025-8 (1978)

432. D. S. King, R. L. Sharp, M. D. Vukovich, G. A. Brown, T. A. Reifenrath, N. L. Uhl and K. A. Parsons: Effect of oral androstenedione on serum testosterone and adaptations to resistance training in young men: a randomized controlled trial. Jama 281, 2020-8 (1999)

433. J. D. Peck, B. S. Hulka, C. Poole, D. A. Savitz, D. Baird and B. E. Richardson: Steroid hormone levels during pregnancy and incidence of maternal breast cancer. Cancer Epidemiol Biomarkers Prev 11, 361-8 (2002)

434. L. A. Mucci, P. Lagiou, R. M. Tamimi, C. C. Hsieh, H. O. Adami and D. Trichopoulos: Pregnancy estriol, estradiol, progesterone and prolactin in relation to birth weight and other birth size variables (United States). Cancer Causes Control 14, 311-8 (2003)

435. F. S. vom Saal and F. H. Bronson: Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 208, 597-9 (1980)

436. G. Pointis, B. Rao, M. T. Latreille, T. M. Mignot and L. Cedard: Progesterone levels in the circulating blood of the ovarian and uterine veins during gestation in the mouse. Biol Reprod 24, 801-5 (1981)

437. L. Jansson, T. Olsson and R. Holmdahl: Estrogen induces a potent suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice. J Neuroimmunol 53, 203-7 (1994)

438. H. Offner, K. Adlard, A. Zamora and A. A. Vandenbark: Estrogen potentiates treatment with T-cell receptor protein of female mice with experimental encephalomyelitis. J Clin Invest 105, 1465-72 (2000)

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.

Key Words: Estradiol, Estriol, Progesterone, Testosterone, Neuroinflammation, EAE, Experimental Allergic Encephalomyelitis, TMEV, Th1, Th2, Autoimmune disease, Dendritic cell, Treg, Review

Send correspondance to: Arnaud Nicot, UMR S 546, Faculte de medecine Pierre et Marie Curie-Paris6 site Pitie, porte 310bis, 105 bvd de l'Hopital, Paris, F-75013 France, Tel.: 33 1 40 77 96 84, Fax: 33 1 40 77 81 17, E-mail: anicot @ chups.jussieu.fr

http://www.bioscience.org/current/vol14.htm