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Sex-based differences in autoimmune diseases

Elena Ortona[1], Marina Pierdominici[1], Angela Maselli[2], Caterina Veroni[1], Francesca Aloisi[1] and Yehuda Shoenfeld[3]

[1]*Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, Rome, Italy*
[2]*Dipartimento del Farmaco, Istituto Superiore di Sanità, Rome, Italy*
[3]*Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center (affiliated to Tel-Aviv University), Tel-Hashomer, Israel*

Abstract
Autoimmune diseases are characterized by an exaggerated immune response leading to damage and dysfunction of specific or multiple organs and tissues. Most autoimmune diseases are more prevalent in women than in men. Symptom severity, disease course, response to therapy and overall survival may also differ between males and females with autoimmune diseases. Sex hormones have a crucial role in this sex bias, with estrogens being potent stimulators of autoimmunity and androgens playing a protective role. Accumulating evidence indicates that genetic, epigenetic and environmental factors may also contribute to sex-related differences in risk and clinical course of autoimmune diseases. In this review, we discuss possible mechanisms for sex specific differences in autoimmunity with a special focus on three paradigmatic diseases: systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.

Key words
- sex
- autoimmunity
- systemic lupus erythematosus
- rheumatoid arthritis
- multiple sclerosis

INTRODUCTION

Autoimmune diseases include more than 80 chronic disorders that affect nearly 5% of the population in Western countries. They are characterized by an exaggerated immune response leading to damage and dysfunction of specific or multiple organs and tissues. The aetiology of autoimmune diseases is still unknown, but the available evidence points to an interaction between genetic, environmental and life style factors in disease development. Autoimmune diseases are typically more prevalent in women than in men and are considered the fourth leading cause of disability for women [1]. The strongest sex bias is observed in Sjogren's syndrome, systemic lupus erythematosus (SLE), autoimmune thyroid disease and scleroderma where the ratio of women to men is 7:1-10:1. In rheumatoid arthritis (RA), multiple sclerosis (MS) and myasthenia gravis, the female to male ratio is 2:1-3:1, while there is little or no sex bias in inflammatory bowel diseases and type 1 diabetes [2-4]. Symptom severity, disease course, response to therapy and overall survival may also differ between males and females with autoimmune diseases. In general, women mount stronger humoral and cellular immune responses than men and this is believed to have an effect on the different susceptibility to develop autoimmune diseases. The main factors affecting the differences between female and male immune systems are the sex hormones, the presence of two X chromosomes versus one X and one Y chromosome, and the different response to environmental factors, such as microbial exposure and diet [5]. Sociological differences between genders may also affect the development of autoimmunity. However, the discussion of this topic is beyond the scope of this article and readers can refer to recent reviews [6]. Here, we briefly review the role of sex-related factors in autoimmunity and then highlight key discoveries and controversies on the role of sexual dimorphism in three paradigmatic autoimmune diseases: SLE, RA and MS.

SEX HORMONES IN AUTOIMMUNITY

Due to the presence of hormone receptors on immune cells [7], sex hormones, such as estrogens, progesterone, androgens and prolactin, can influence different aspects of immune system function and potentially affect the risk, activity and progression of autoimmune diseases. Generally, estrogens, in particular 17-β estradiol (E2) and prolactin, act as enhancers at least of humoral immunity, and testosterone and progesterone as natural immunosuppressants [3]. Sex hormones have different effects depending not only on the concentration but also on the type of target cell and the receptor subtype expressed on a given cell type. At periovulatory to pregnancy levels [8], E2 has mainly anti-inflammatory effects, by inhibiting production and signaling of pro-inflammatory cytokines, such as tumor

Address for correspondence: Marina Pierdominici, Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: marina.pierdominici@iss.it.

necrosis factor (TNF), interleukin (IL)-1β and IL-6, and natural killer (NK) cell activation, and by inducing expression of anti-inflammatory cytokines favoring a T helper 2 (Th2) phenotype [9], such as IL-4, IL-10 and transforming growth factor (TGF)-β, and by activating regulatory T cells (Treg) [10]. At lower concentrations, E2 stimulates TNF, interferon (IFN)-γ, IL-1β and NK cells, while it enhances antibody production by B cells both at high and low concentrations [9]. Prolactin increases antibody production, regulates the development of CD4+ T cells and triggers pro-inflammatory cytokine production [11]. Progesterone stimulates a switch from a predominantly pro-inflammatory to an anti-inflammatory immune response, favors Treg differentiation [12], and exerts an inhibitory effect on NK cells. Several studies indicate that testosterone has suppressive effects on the immune system by inhibiting pro-inflammatory cytokine production, Th1 differentiation, immunoglobulin production and NK cell cytotoxic activity, and by potentiating the expression of anti-inflammatory cytokines [13].

Consistent with the effects of sex hormones on immunity, changes in the severity of autoimmune diseases are observed during pregnancy, when estrogens and progesterone reach the highest levels [14]. Maternofetal immune tolerance is essential to maintain pregnancy and one of the important adaptations leading to this immune tolerance is the shift, at implantation, from a pro-inflammatory Th1/Th17 response, which promotes rejection, toward a Th2/Treg cell response that promotes tolerance and inhibits NK cell cytotoxicity. Due to these adaptive changes in immune system function, pregnancy has opposite effects on some autoimmune diseases. For instance, pregnancy is associated with an increase in disease flares in SLE, this effect being related to the increased Th2 response and enhanced production of pathogenic autoantibodies [15]. Conversely, pregnancy has a protective effect in Th1-dominant immune diseases, like RA and MS.

SEX CHROMOSOMES IN AUTOIMMUNITY

The female karyotype includes two X chromosomes, one derived from each parent, while men carry one maternal X and one paternal Y chromosome. To avoid double dosage of X chromosome-derived proteins, one of the X chromosomes is randomly silenced in females in the early stages of embryogenesis. However, X chromosome inactivation is not complete and about 15% of the genes escape inactivation, leading to overexpression of some X-linked genes in females [16]. The X chromosome encodes several immune-related genes, such as CD40 ligand, chemokine receptor CXCR3, O-linked N-acetylglucosamine transferase, forkhead box P3(FOXP3), toll-like receptor (TLR)7, TLR8, IL-2 receptor gamma, tyrosine-protein kinase BTK, and IL-9 receptor, whose overexpression may influence the immune response in a sex-dependent manner [5]. An important role for genes on the partially inactivated X chromosome in immune system function is highlighted by the finding that both the absence of a normal second X-chromosome in females (Turner syndrome) and the presence of two or more X chromosomes in males (Klinefelter syndrome) are associated with an altered susceptibility for some autoimmune diseases as compared with sex-matched general population (*e.g.*, SLE prevalence is decreased in Turner syndrome and increased in Klinefelter syndrome) [17, 18]. Experimental studies suggest that the Y chromosome may play a protective role in the development of autoimmunity [19].

SEX DIFFERENCES IN microRNA EXPRESSION IN AUTOIMMUNITY

MicroRNAs (miRNAs) are short non-coding RNAs (19-24 nucleotides in length) that regulate gene expression mainly at the post-transcriptional level by binding to the 3' UTR of target genes and inducing translational inhibition or degradation of the target mRNA. Their expression and function are essential for the development of different physiological systems and the maintenance of cell homeostasis. Because miRNAs are critical for the development, differentiation and function of both innate and adaptive immune cells, dysregulation of their expression may contribute to the development of autoimmune diseases [20]. It has been reported that miRNAs are differentially expressed in males and females in both gonadal and non-gonadal tissues, but it is not clear yet what drives this sex-related differential expression. The X chromosome is highly enriched in miRNAs whereas only two miRNAs, at least in humans, have been assigned to the Y chromosome thus far [21]. Although most of the X-linked miRNAs have no known function, some of them are reported to play a role in immunity or autoimmunity [22, 23]. The presence of a second X chromosome in females can affect miRNA expression levels and this may be crucial for the development of female biased-autoimmunity. Interestingly, E2 can regulate miRNA expression in different cell types and tissues, by both genomic and non-genomic mechanisms of action [24], while miRNAs regulate E2-dependent signaling by targeting proteins and signaling molecules that are involved in estrogen signaling [22]. Exploring miRNAs in the context of sex-biased autoimmune disorders may provide novel insights into disease pathogenesis and lead to the identification of new therapeutic targets.

SEX DIFFERENCES IN THE GUT MICROBIOTA

It is well established that gut microbiota (*i.e.*, the collection of bacteria, viruses, fungi and protozoa lining the gastrointestinal mucosa) affects innate and adaptive immune responses. On the other hand, the immune system influences the composition of the gut microbiota and this interaction can have important consequences for the development of inflammatory diseases [25]. A role for gut microbiota in the sex bias in autoimmunity has been revealed by different studies in animal models. For instance, in the spontaneous NOD model of type 1 diabetes characterized by a strong female bias, disease rate was similar in germ-free (GF) female and male NOD mice, suggesting that male-specific microbiota play a protective role [26]. This effect could be mediated, at least partially, via microbiota metabolism of sex

hormones. In fact, gavage of female NOD weanlings with male NOD-derived intestinal microbiota resulted in elevated testosterone levels and type 1 diabetes protection as compared with unmanipulated females [26]. Conversely, sex hormones may affect gut microbiota because sex-specific differences in the composition of the microbiota are found only after puberty [26, 27]. Similarly to what observed in NOD mice, the composition of gut microbiota in mouse models of lupus and RA has been found to be significantly different in male and female adult mice [28, 29]. Summarizing, specific gut microbiota patterns appear to be associated with autoimmunity. However, the role of gut microbes and their interactions with hormones in the sex bias in autoimmunity is still poorly characterized and no data are available in humans. Further studies are needed to elucidate the specific mechanisms and/or molecules produced by gut commensals that are affected by and affect sex hormones, and may be involved in the protection from autoimmune diseases.

OTHER FACTORS POTENTIALLY INVOLVED IN THE SEX BIAS IN AUTOIMMUNITY

Microchimerism. Maternal and fetal cells are exchanged during pregnancy, leading to fetal cell persistence in the mother (microchimerism). Chimeric fetal cells are often hematopoietic and can differentiate into somatic cells in different organs, becoming a potential target for autoimmunity [30]. The available data on the role of fetal microchimerism in autoimmunity are weak or inconclusive.

Environmental estrogens. In addition to endogenous estrogens, the immune system can be targeted by natural (phytoestrogens and mycoestrogens) or synthetic (xenoestrogens) compounds with estrogenic activity, *i.e.*, estrogenic endocrine disruptors [31]. Xenoestrogens can be present in cosmetics and personal care products (makeup, hair dyes, soaps, perfumes) more commonly used by women. The actual impact of both endogenous and environmental estrogens on the immune system is still under investigation. Environmental estrogens may display a synergic/additive effect with endogenous estrogens potentially affecting the immune response. The influence of oral contraceptives and hormone replacement therapy on the risk and progression of autoimmune disease in women also deserves consideration.

SYSTEMIC LUPUS ERYTHEMATOSUS

SLE is a multifactorial and highly polymorphic systemic autoimmune disease that affects multiple organs including kidneys and heart [32]. The incidence of the disease is estimated at 20–50 cases/100 000 individuals. To date, the etiology of SLE remains unknown; however, it is likely that a complex interaction between genetic, environmental (*e.g.*, infectious agents, including Epstein-Barr virus and parvovirus, UV light, drugs, cigarette smoking and silica dust), and hormonal factors promotes the immune dysfunction leading to the disease. SLE is characterized by autoantibody production by dysregulated B cells, target organ infiltration by inflammatory T cells and aberrant immune cell activation. The autoantigen-autoantibody interaction triggers the formation of immune complexes that, once deposited, cause tissue injury [33].

SLE is often called a "woman's disease" because of the striking differences in prevalence related to sex. Pre-menopausal women have SLE incidence rates of 8:1-15:1 when compared to age-matched males; these rates decline to 3:1 in the pre-adolescent population and to 5:1 after menopause, when estrogen levels are more similar between genders. Clinical observations and experimental data clearly indicate that E2 influences the development of SLE [3, 34]. Studies in lupus experimental models have demonstrated that E2 promotes disease via estrogen receptor (ER) α activation [35] whereas ERβ activation has mild immunosuppressive effects [36]. ERα-deficient mice show significantly less aggressive renal disease and proteinuria and survive better than wild-type mice [37]. Importantly, an accelerated metabolic conversion of androgen precursors to E2, due to aromatase activation, has been observed in SLE, partially explaining the increased availability of E2 in this disease [38]. Moreover, the identification of autoantibodies reacting with ERα in SLE patients and their correlation with disease activity [39] have disclosed a new research area in estrogen-related effects in autoimmunity. Results from different studies indicate that the use of oral contraceptives and hormone replacement therapy increases the risk of developing SLE; however, some retrospective studies suggest no increase in clinical flares with hormonal therapy [40]. Importantly, a reduction in disease activity has been observed in SLE patients treated with the pure ER antagonist Fulvestrant (Faslodex) [41].

X-linked genes, such as *FOXP3, TNF* and *TLR7*, have been associated with gender bias in SLE [5]. Additionally, several X-linked miRNAs have been found to be upregulated in CD4+ T cells from female SLE patients compared to male patients [22], potentially contributing to the sex bias in SLE. E2 may contribute to the gender bias in SLE by modulating expression of selected miRNAs [42]. miR146a, which is a negative regulator of the IFN-α pathway, and miR125a, which negatively regulates the inflammatory chemokine RANTES, are profoundly decreased in peripheral blood mononuclear cells from patients with SLE as compared to healthy donors [43, 44]. Conversely, miR148a, which contributes to DNA hypomethylation in lupus CD4+ T cells, has been found up-regulated [45]. In splenocytes from estrogen-treated mice, miR148a was up-regulated whereas miR146a and miR125a were down-regulated [46].

In males, SLE has a late onset and different clinical features and outcomes, suggesting that male-specific predisposing and/or pathogenetic factors exist. To date, there is limited evidence to suggest an altered hormonal milieu in men with lupus [47, 48]. Potential risk factors include X-chromosome abnormalities (as supported by the increased incidence of SLE in patients with Klinefelter syndrome) and various somatic genetic polymorphisms. Further studies are required to understand the sex-related aspects of SLE disease susceptibility, clinical features and outcome, potentially providing new tools for clinical intervention. Sex-specific factors affecting SLE are summarized in *Table 1*.

Table 1
Sex specific factors affecting systemic lupus erythematosus

Hormones	Accelerated metabolic conversion of androgen precursors to E2 (aromatase activation) [38] E2 effects on immune function [3, 34]
Genetic factors	X-linked genes (FOXP3, TNF, TLR7) [5]
Epigenetic factors	X-linked miRNAs [22] Estrogen up-regulated miRNA (miR148a) [22] Estrogen down-regulated miRNA (miR146a, miR125a) [46]
Clinical phenotype	Incidence of Raynaud's phenomenon, alopecia, malar rash and arthralgia/arthritis higher in females than in males [47]

RHEUMATOID ARTHRITIS

RA affects approximately 1% of the general population and is characterized by chronic joint inflammation, functional impairment, disability, and premature mortality. It is widely accepted that RA is caused by various environmental factors in genetically predisposed individuals. RA is about three times more common in women than in men, with a peak age of onset in the fifth decade of life. The female to male prevalence ratio is around 2:1 in 55 to 64-year-olds, shifting to a male excess in people over 75-years old. Data concerning female sex hormone exposure and RA risk are conflicting [49]. The higher prevalence of RA in females than males, at least before 75 years of age, could suggest that E2 and progesterone increase the risk of disease. However, as stated above, RA presents more often after menopause, and past large cohort studies did not establish any relationship between oral contraceptives or hormone replacement therapy and the risk of developing RA [40]. Nulliparity is associated with an increased risk of disease development whereas pregnancy seems to decrease it [50]. These data show, pregnancy effects apart, that the female to male ratio observed in RA probably involves factors other than E2 and progesterone. Regarding disease activity, whereas in animal models of RA female sex hormones have beneficial effects, their role in humans is less clear as both pro- and anti-inflammatory effects have been described. On the one hand, higher disease activity scores have been reported in women as compared to men [51]. Additionally, estrogens are significantly elevated, relatively to androgens, in synovial fluid from both male and female RA patients; this is due to high aromatase activity that is induced by locally produced inflammatory cytokines, favoring macrophage and fibroblast proliferation and therefore the inflammatory process [52]. On the other hand, the clinical features of RA are ameliorated in approximately 75% of pregnant patients who have high serum levels of E2 and progesterone [53]. Notably, relapses are frequent in the postpartum period when the levels of these hormones fall [54]. Clinical trials examining the effects of oral contraceptives or hormone replacement therapy on the severity or progression of existing RA in postmenopausal women have yielded contrasting results, some studies showing evidence of a beneficial effect of estrogens and other studies finding no effect [40]. Finally, active RA is characterized by an activity peak early in the morning which correlates with prolactin plasma levels [55].

Regarding male sex hormones, the available data suggest that the onset and severity of RA are inversely associated with androgen levels, providing a possible explanation for the increased rate of RA incidence in men after 55 years and for the less severe disease course in men as compared to females [56, 57]. Men also have a better response to RA therapy than women [58]. Interestingly, there is evidence that some RA patients of both sexes have reduced amounts of serum androgens already several years before disease onset. In particular, female RA patients have lower than normal levels of dehydroepiandrosterone and/or dehydroepiandrosterone sulfate. Androgen replacement therapy has positive effects in male RA patients, particularly as adjuvant treatment [59], and a slight disease-modifying effect, not statistically significant, in postmenopausal women with RA [60]. There is incomplete information on genetic influences on sex disparities in RA. Recently, polymorphisms in the CYB5A gene, which is relevant for androgen synthesis, have been found to be associated with risk of RA in women, but not men, thus contributing to the sex bias observed in RA [61]. In contrast to SLE, RA has been described very rarely in patients with Klinefelter syndrome, suggesting that the extra X chromosome does not confer an added risk for RA. One study found an association between RA and single nucleotide polymorphisms (SNP) of the X-encoded genes TIMP1 (which inhibits matrix metalloproteinases and prevents cartilage degradation) and IL-9 receptor (which is involved in IL-9 signaling and in early T cell development) [62]. To date, no data are available on epigenetic modification of the inactivated X chromosome in relation to RA susceptibility. Sex-specific factors affecting RA are summarized in *Table 2*.

MULTIPLE SCLEROSIS

MS affects 1 in 1000 people in Western countries and is the most common chronic inflammatory disease of the central nervous system causing neurological disability. MS is usually diagnosed in young adults and characterized by bouts and remissions followed by a secondary progressive course. Less frequently, MS has a progressive course right from the onset. MS affects women two to three times as often as men. While females are at higher risk for MS, males are more likely to display primary progressive disease and accumulate disability faster than female patients in relapse-onset MS [63]. The sex ratio in MS appears to be rising; this trend is

Table 2
Sex specific factors affecting rheumatoid arthritis

Hormones	Estrogen effects on immune function (both pro-inflammatory and anti-inflammatory effects, induction of Tregs) [50] Progesterone effects on immune function (anti-inflammatory effects, induction of Tregs) [50] Androgen effects on immune function (anti-inflammatory effects) [57] High aromatase activity in synovial fluid (® prevalence of synovial estrogens relative to androgens) [52]
Genetic factors	Single nucleotide polymorphisms of the CYB5A gene in RA females [61] Single nucleotide polymorphisms of the X-encoded genes *TIMP1* and *IL-9R* [62]
Clinical phenotype	Less severe course of illness and better response to therapy in males as compared to females [56, 58] Amelioration of RA in pregnant females [53]

noted primarily in relapsing-remitting MS and is associated with a latitudinal gradient [64].

As for other autoimmune diseases, the cause of MS is not well understood but a complex interaction between genetic and environmental factors is clearly involved. The HLA-DR2 allele within the MHC region is the major genetic risk factor for MS [65]. In recent MS genome wide association studies, 110 variants have been identified outside the HLA region, which are typically common, mostly related to immune system function and have very modest effects on disease risk [66]. To date, none of the studies of HLA and non HLA genes have convincingly shown that genetic differences between women and men affect MS susceptibility. Also, no MS susceptibility loci are confirmed to be located on the X chromosome.

The role of hormonal changes in the modulation of MS risk and course in female patients has been extensively investigated. Puberty and pregnancy have received much attention in MS research, while the impact of breastfeeding, oral contraceptives use, menopause and HRT on MS risk and course is less understood. After puberty there is a dramatic, female-specific increase in MS risk; earlier puberty and obesity during childhood are significant risk factors for MS [67]. Female MS patients experience clinical improvements during pregnancy, particularly in the last trimester, with a rebound in relapses occurring in the first trimester postpartum [68]. Specific changes in the expression of a limited number of immune-related genes during pregnancy were found associated with a decrease in MS disease activity assessed by occurrence of relapses during pregnancy [69]. Testosterone, progesterone and estriol, the major estrogen during pregnancy, have been found to induce anti-inflammatory as well as neuroprotective effects in preclinical models of MS, suggesting the potential use of these hormones for the treatment of MS [70]. Lower testosterone levels have been associated with higher disability in men with MS [71]. The results of two phase 2 trials of transdermal testosterone in male MS patients [72] and oral estriol in female MS patients [73] are encouraging but warrant further investigation in phase 3 trials.

Findings of an increasing sex ratio in MS implicate heightened female responsiveness to changing cultural and environmental cues. Vitamin D deficiency, Epstein-Barr virus infection and smoking history are known to influence MS risk [74]. Recent studies indicate that cigarette smoking is also a risk factor for disease progression [75]. However, it is still poorly understood how environmental factors interact with hormones, metabolic factors, including diet and/or altered gut microbiota, and a susceptible genetic background to shape MS. Sex-related differences in neuroimaging features [76], cerebrospinal and peripheral blood biomarkers [77, 78] and effectiveness of first- and second-line therapies for MS need to be thoroughly explored to yield new insights into MS pathological mechanisms and help in treatment decisions in the growing armamentarium of drugs that can ameliorate MS. Sex-specific factors affecting MS are summarized in *Table 3*.

CONCLUSIONS

Differences in prevalence and severity of autoimmune diseases between males and females result from complex and still poorly understood interactions between genetic, hormonal and environmental factors. The increasing use of multi-omics and bioinformatic approaches in large and clinically well characterized patient cohorts will help identify critical pathways and

Table 3
Sex specific factors affecting multiple sclerosis

Hormones	Female specific increase in MS risk after puberty [67] Neuroprotective and anti-inflammatory effects of testosterone, progesterone and estriol in MS-like disease models [70] Dramatic decrease in MS relapse frequency during the second half of pregnancy, followed by a rebound in relapses in the first trimester postpartum [68]
Genetic factors	Still unknown
Clinical phenotype	Faster accumulation of disability in male than female patients in relapse-onset MS [63]

networks that can be useful for the discovery of new biomarkers and targeted for sex-specific therapeutic intervention and/or prevention of autoimmune diseases.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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