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EXPERT OPINION

Sex bias in immune response: it is time to include the sex variable in studies of autoimmune rheumatic diseases

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Abstract

Healthy females and males differ in their immune cell composition and function and females generally mount stronger immune response than males and are much more susceptible to autoimmune rheumatic diseases. Females differ from males in sex hormones, and X-chromosome genes. Sex hormones affect immune cells and responses, and may induce epigenetic DNA changes. The importance of X-chromosome genes is exemplified in men with the Klinefelter syndrome (47,XXY) who have an additional X-chromosome and develop systemic lupus erythematosus(SLE) as frequently as women. X-chromosome contains genes critical for the immune response, such as *FOXP3*, *toll-like receptor(TLR)7*, *TLR8*, *CD40 Ligand*, *IL2RG, IL9R, BTK*, and others. Whereas one X-chromosome in females is randomly inactivated early in embryonic development, around 25% of X-linked genes escape inactivation and result in more X-linked gene dosage in females. We use two key female-biased autoimmune rheumatic diseases, SLE and systemic sclerosis, to review differences in immune response, and clinical manifestations between females and males. The inclusion of sex variable in research will facilitate precision medicine and optimal patient outcome.

Keywords Bias · Difference · Female · Male · Sex · Precision medicine systemic sclerosis · Systemic lupus erythematosus

Introduction

For years, it has been acknowledged that females and males differ in their immune responses. For instance, antibody responses to influenza vaccine are much stronger in women than men [1]. This comes at a price. Women are more susceptible to autoimmune diseases. Nine out of 10 individuals who develop systemic sclerosis (SSc), systemic lupus erythematosus (SLE), or Sjogren's syndrome (SjS) syndrome are women [2–4]. In a large and longitudinal study of patients with type I diabetes mellitus in USA the prevalence of additional autoimmune diseases was 1.9-times greater in women than men [5].

There are many sex differences between healthy females and males in immune cell composition and function at the innate, adaptive (cellular and humoral) levels and these can be found in excellent reviews [6]. Females have higher proportions of CD4 + T cells, CD19 + B cells and plasma cells, but lower proportion of monocytes and natural killer (NK) cells than men [6, 7]. In addition, females exhibit greater innate, humoral and cellular responses than males [1, 6]. Many of the differences between females and males in immune response may partly attributed to differences in sex hormones, and X chromosomes.

We chose to concentrate on two key female-biased autoimmune rheumatic diseases (AIRDs), SLE and SSc, with diverse clinical manifestations [8, 9]. We searched the Pubmed using the terms sex difference and autoimmune disease, systemic sclerosis, systemic lupus erythematosus. We also used references from retrieved articles. Our purpose was to highlight differences between females and males in immune responses which will help researches incorporate

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sex variable in future studies to achieve homogeneous groups of patients with ultimate goal personalized medicine.

Sex hormones

The increased incidence of autoimmune rheumatic diseases (AIRDs) during the reproductive years suggests that sex hormones are implicated. For instance, female to male ratio in SLE prior to puberty is 2:1 and after puberty is 9:1 re-enforces the concept that estrogen contributes to SLE susceptibility [10]. On the other hand, male sex hormones are protective in lupus-prone NZB/NZW mice. Male mice develop lupus-like disease later than female mice but castrated males develop disease at the same time as females [11, 12].

Sex steroid hormones exert multiple and variable effects on many immune cells depending on cell type, and the experimental system. In general, estrogen has immunostimulatory effects on adaptive immune system, including an in vivo increase of Ig-producing plasma cells, although it also increases human Tregs numbers and function, whereas androgens have immunosuppressive effects [12–14]. Estrogen also increases the production of prolactin which has immunostimulatory effects promoting the production of CD40 Ligand (CD40L), IL-6, type I interferon α (IFNα) and T cell proinflammatory cytokines [15]. Estrogen, through estrogen receptors (ERs), stimulates IL-6 expression in mice and humans [16] and, by inhibiting apoptosis of autoreactive T cells, may contribute to autoimmunity in SLE [13]. Interferon regulatory factor 5(IRF5) that controls the expression of type I IFN, a significant pathogenic factor for SLE and SSc [17, 18], was upregulated by estrogen in vitro in mice [19]. In addition, TLR7-mediated IFNα production by plasmatoid dendritic cells (pDCs) is increased in females compared to males and this is positively regulated in vivo by estrogen through estrogen receptor signaling [20]. Unc-93 homolog B1(UNC93B1), an endoplasmic reticulum transmembrane protein, essential for the function of TLR7 and TLR9 was increased by estrogen in mice [21].

Estrogen, depending on cell type and sex exhibit variable effects on peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor, and a master regulator of adipocyte differentiation. PPARγ expression is higher in female than male T cells, and is enhanced by estrogen in male T cells, but is inhibited by estrogen in cancer cells [22, 23]. PPARγ is also involved in immune cell modulation as it drives macrophage M2 differentiation, inhibits TH1 and TH17 cells [24], and is necessary for Tregs function [25]. In addition, macrophage PPARγ is required for phagocytosis of apoptotic cell bodies, whereas macrophage PPARγ knock-out mice developed lupus-like glomerulonephritis [26]. PPARγ also regulated TGFβ-mediated fibrogenesis and protected from PAH in mice [27]. Pioglitazole, a PPARγ agonist, significantly decreased activation of CD4 + T cells from female mice during the estrus stage of menstrual cycle, but not CD4 + T cells during the diestrus stage of menstrual cycle or CD4 + T cells from male mice. In addition, pioglitazone treatment reduced T follicular helper (TFH) responses in female but not in male mice [28], whereas female, but not male, CD4-PPARγ knock-out mice had increased TFH cells and germinal center B cells and spontaneously developed autoimmune phenotypes [28].

Sex hormones can induce epigenetic changes. For instance, estrogen, by downregulating DNA methyl-transferase 1 expression that leads to DNA hypomethylation in T cells from SLE female patients may contribute to autoimmunity in SLE [13]. In transgender individuals, an *IL21* promoter-associated region was demethylated at 12-month post-feminizing hormonal therapy (estrogen in combination with anti-androgen) but gained DNA methylation at 12-month post-masculinizing hormonal therapy (testosterone)[29]. miRNAs are different between female and male lupus-prone mice, whereas estrogen promotes lupus-related miRNAs in castrated mice [30]. miRNAs were also found to have a pathogenic role in SSc and SLE [31–33].

Gut microbiota may alter sex hormone levels and autoimmune disease susceptibility. Markle et al. [34] showed in the non-obese diabetic (NOD) mice, a model of type I diabetes mellitus, that female mice under germ-free conditions no longer had the increased susceptibility to disease than males. In addition, microbiota transfer from adult NOD male mice to immature females increased testosterone levels and protected against diabetes through androgen receptor [34]. There are excellent reviews on sex hormones in AIRDs [35, 36].

X chromosomes

Apart for sex hormones, chromosome genes are critical for immune responses. Individuals with Klinefelter syndrome (47,XXY) develop more frequently SLE. The risk of SLE in men with Klinefelter syndrome (47,XXY) is increased by 14-fold compared to men (46,XY) and is similar to that in women (46,XX) [37] and this implies susceptibility due to X chromosome gene-dose effect. The importance of X-chromosome genes is supported by the 'four core genotype'(FCG) mouse model. This model involves a deletion of a testis-determining *Sry* gene from the Y chromosome which results in gonadal female XY-mice, and an insertion of *Sry* transgene onto an autosome which results in gonadal male mice. The four genotypes created when XY-*Sry* gonadal male mice mate with XX female mice are: XX mice with ovaries, XX*Sry* mice with testis, XY-mice with ovaries and XY*Sry* mice with testis [38]. This model allows the evaluation of the effects of sex hormones and the effects

of X-chromosome complement. Gonadectomized FCG mice exhibited increased susceptibility to pristine-induced lupus in XX mice suggesting that an additional X-chromosome confers susceptibility irrespective of female gonads [38].

In recent years, much progress has been made towards deciphering the mechanisms of X chromosome-mediated susceptibility to autoimmune diseases. Females have two copies of X chromosomes, one paternal, one maternal, while males have only one copy, and this would have led to genes dosage imbalance between the sexes. One X chromosome is randomly inactivated early in embryonic development in females to maintain X-linked genes balance between sexes. However, 20–30% of X-linked genes escape inactivation in humans [6, 39].

X chromosome inactivation is maintained in mature T cells but is disrupted in T cells of SLE patients which exhibited abnormal upregulation of X-linked genes [40]. X chromosome inactivation was also disrupted in T cells of late-stage disease of female NZB/W F1 SLE mouse model [40]. Skewed X-chromosome inactivation has also been reported in peripheral blood cells from women with SSc [41]. More importantly, skewed X-chromosome inactivation in females patients with SSc was associated with decreased expression of *FOXP3* [42].

Various important immune-related genes reside on X chromosome, including *FOXP3*, *toll-like receptor (TLR)7*, *TLR8*, *CD40L*, cytokine receptors (*IL13RA2, IL2RG, IL9R*), *BTK*, and *KDM6A* (encoding UTX, an epigenetic regulator with demethylase activity)[43]. FOXP3 is a crucial transcription factor for the suppressive function of Tregs, as loss-of-function mutations of *FOXP3* gene results in the X-linked immune dysregulation, polyendocrinopathy, enteropathy (IPEX) syndrome, characterized by type I diabetes mellitus (DM), autoimmune thyroiditis (Hashimoto), Addison's disease, enteropathy, dermatitis, and other immune manifestations, including thrombocytopenia, and hemolytic anemia [44]. Polymorphisms of *FOXP3* gene were detected in various autoimmune diseases, including rheumatoid arthritis, SLE and type I DM. In a recent study UTX, which enhances NK effector function, was found to escape X chromosome inactivation and was expressed more in females than males. For instance, male NK cell IFNγ production was decreased independently of gonadal hormones, whereas IFNγ, as well as granulocyte monocyte–colony-stimulating factor (GM–CSF) production, was reduced in female NK cells with one UTX copy number [45]. In addition, *TLR7* gene escapes silencing by X chromosome inactivation in B cells, monocytes, and plasmacytoid dendritic cells (pDCs) from women and from Klinefelter syndrome males and this resulted in greater *TLR7* expression and greater B cell response to T cells [46]. TLR7 senses endosomal ssRNA originating from extracellular self-nucleic acids which derive from defective clearance of apoptotic bodies and is involved in autoimmune germinal center formation and autoantibody production [47]. Expression of *TLR7* was higher in XX and XXY relative to XY immune cells, including pDCs, B cells and monocytes [46]. TLR7-mediated IFNα production by pDCs is increased in SLE [48], whereas overexpression of *TLR7* in mice resulted in SLE development [46, 49]. TLR7 also enhanced profibrotic tissue inhibitor of metalloproteinase 1(TIMP-1) in SSc monocytes [50]. One study reported that both X-chromosome gene dosage and estrogen receptors signaling contribute to enhanced TLR7-mediated IFNα production in pDCs from women [51]. A *TLR8* transgenic mouse model of SSc exhibited more severe skin fibrosis which was abrogated by pDC deletion [52]. TLR8 was upregulated in pDCs in SSc [52]. CD40L, a co-stimulatory molecule on CD4 + T cells, was found to be overexpressed in T cells from female patients with SLE [53] and SSc [54].

X chromosome genes may also have more generalized effects than susceptibility to autoimmune diseases. For instance, in a four-core genotype mouse model, it has been demonstrated that XX mice with either testes or ovaries live longer than XY mice with either testes or ovaries [55].

Autosomal genes

While autosomal genes are not different between females and males, their expression may differ, and differences in gene expression on autosomes are conserved among mamalians [56, 57]. Transcriptomics analysis of T cells and B cells from healthy human identified hundreds of sex-biased autosomal transcripts [58]. In addition, after stimulation of peripheral blood leukocytes with lipopolysaccharide (LPS), an innate stimulation, sex-specific responses were found in hundreds of autosomal genes [59]. Sex dimorphic gene expression throughout the genome was also detected in mouse neutrophils [60]. Furthermore, there was a remarkable cell specificity in sex-biased gene expression. Transcriptome analysis of untreated immune cells from C56BL/6 mice, showed that sexual dimorphism was restricted to macrophages and mediated by innate immune pathways [61]. In addition, Mendelian randomization analysis showed an association of *IL6* with rheumatoid arthritis(RA) in females but with psoriatic arthritis in males [62].

Environment exerts different effects on gene regulation in females and males. For instance, female mice exhibited differences in gene expression compared to male mice fed identical diet [63].

There are excellent reviews on the sex dimorphism in immune cell composition and function, and X-chromosome inactivation-related immune effects [6, 64, 65].

Sex bias in clinical manifestations

Environmental factors confer different susceptibility to AIRDs. In a meta-analysis, silica exposure confers increased susceptibility for SSc to males, but not to females [66]. As already mentioned, women develop AIRDs much more frequently than men. However, men develop more severe manifestations of AIRDs than women. In a systematic review, male sex was found to be is a risk factor for rheumatoid arthritis-associated interstitial lung disease (RA–ILD) [67]. In a meta-analysis renal involvement, serositis and thrombocytopenia were more frequent in male SLE than femal SLE patients [68]. Men's suffering from serious disease manifestations is best illustrated in SSc. In a review, men were more likely to develop scleroderma renal crisis, diffuse cutaneous SSc (dcSSc), anti-topoisomerase I antibodies (ATA), and more active disease [69]. In addition, in SSc-associated pulmonary arterial hypertension (PAH) male sex was strongly associated with rapid disease progression [70]. In addition, males were predominantly ATA-positive compared to females, and PAH and dcSSc were more frequent in males after adjusting for autoAb status [71]. In a randomized controlled trial of SSc-ILD patients treated with mycophenolate mofetil (MMF) or oral cyclophosphamide, men had worse radiographic progression at 2 years compared to women after adjusting for baseline disease severity and treatment received, and worse survival [72]. In addition, mortality, the hard outcome of a disease was high in men with SSc. In two large inception cohorts of SSc patients a multivariable analysis showed that male sex was associated with 5-year mortality [73]. In a 10-year study of very large cohorts, the Leiden combined care in SSc (CISS) and the European scleroderma trials and research(EUSTAR), multivariable analysis showed that male sex was the most important risk factor for all-cause mortality of SSc patients [71]. SSc-related mortality was higher in men than women in the Norwegian cohort [74] and in the EUSTAR cohort [71]. Sex bias in mortality in infectious diseases also reported. An analysis of COVID-19 data from many countries revealed a male bias in mortality [75].

Sex bias in response to immunotherapy

Given the sex difference in immune response one would expect differences to pharmacological treatments between females and males regarding both immunotherapy efficacy and adverse effects.

In TNFα-naïve spondyloarthritis (SpA) patients, men discontinued adalimumab more frequently than women due to inadequate response [76]. In contrast, other investigators found that RA remission, as defined by no swollen joint, no tender joint, and normal erythrocyte sedimentation rate, was significantly lower in women than men at 2 year and 5 year post-treatment [77]. In addition, in a 20-week randomized double-blind, placebo-controlled trial in patients with diabetic cardiomyopathy, a phosphodiesterase 5 inhibitor tadalafil resulted in improvement in cardiac torsion and fiber shortening in men but not in women [78]. The identification of gene expression difference between females and males in SSc is expected to lead to optimal immunotherapy, as gene expression changes reflected clinical response in abatacept- and mycophenolate mofetil-treated patients with SSc [79, 80].

Regarding adverse drug reaction it should be noted that women are more susceptible to drug effects, as they are exposed to higher concentrations and longer elimination times of drugs compared to men, and these pharmacokinetics parameters are associated with higher incidence of adverse drug reactions [81, 82].

Women develop higher ab responses to influenza vaccine but also more frequent adverse effects compared to men [1]. In addition, more women than men experienced adverse effects and anaphylactic reactions to Phizer/BioNTech COVID-19 vaccine [83, 84] and more serious adverse effects to biological treatment in RA [85]. In a post-hoc analysis of pooled data from four randomized controlled trials of Nintedanib in autoimmune disease—ILD, women were more likely to have gastrointestinal tract (GIT) adverse effects, elevation of liver enzymes, drug dose reduction and drug interruption compared to men [86].

Concluding remarks

In conclusion, more and more accumulated data point to differences in innate and adaptive immune responses between females and males. These differences are not restricted to X-linked genes expression but are located throughout the genome. Certain differences between females and males are present during the reproductive years, highlighting the importance of sex hormones [65]. Therefore, sex is a variable that should always be considered in research studies and trials in AIRDs. This approach will help better define disease susceptibility, early diagnosis, homogeneous patient groups, and optimal immunotherapy. Identifying immune-related escape genes and their expression in immune cell subsets will be an important step towards precision medicine.

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Declarations

Conflict of interest Disclosure of potential conflicts of interest: none declared.

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