

A new hypothesis of the possible mechanisms of gender differences in systemic lupus erythematosus

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ABSTRACT

Numerous studies have suggested that sex hormones, especially oestrogens, can contribute to the onset and development of the disease activities of systemic lupus erythematosus (SLE), and this seems to be associated with the gender bias of SLE. In fact, there is significant evidence of the inductive effects of oestrogens on autoimmune-related immune responses, such as the production of antibodies, cytokines, and autoantigens including human endogenous retroviruses (HERV). The higher susceptibility to oestrogens in patients with SLE may be regulated by quantitative/qualitative abnormalities of oestrogen receptors (ERs) and different immune responsiveness to oestrogens in SLE patients in comparison to normal controls. In addition to previous findings, this report reviewed and discussed possible the mechanisms of gender bias of SLE based on results obtained by recently developed technologies such as DNA microarray methods.

Introduction

SLE, which is a representative autoimmune disease, occurs far more frequently in females than in males, and there have been numerous studies addressing this issue in both humans and mouse models (1-6). Sex hormones such as oestrogen seem to be an important factor in this gender difference in the incidence of SLE (4, 7). In fact, oestrogens can play an important role in promoting autoimmune-related immune responses, including the production of cytokines, antibodies, and endogenous autoantigens such as HERV (which is reported to be one of the pathogenic factors of SLE) (8-10). Why are patients with SLE susceptible to the influence of these oestrogen-mediated

immune responses? These are recent studies that suggest a resolution of this issue, e.g. there may be quantitative or qualitative abnormalities of oestrogen receptors, and different immune regulatory responses to oestrogens in the patients with SLE in comparison to healthy controls (11, 12).

The pathophysiological mechanism of SLE may involve a combination of several activating or environmental factors (such as viral and bacterial infection, ultraviolet light and stress) with the predisposition of the host (genetic factors such as human leukocyte antigen; HLA types). Shoenfeld *et al.* referred to the "Mosaic of Autoimmunity" as the combination of factors associated with the induction of SLE (13-15). The recently developed biological technologies for performing comprehensive gene analyses, such as DNA microarray methods, appear to be useful for the pathogenic studies of SLE induced by these complicated factors (12-18). In addition to the previously reported findings, based on recently obtained findings using these new biological methodologies, this report reviews the role of sex hormones, especially oestrogens, in the pathogenesis of SLE and discusses the possible mechanism of gender bias in the incidence of SLE.

Gender discrepancy in SLE and roles of sex hormone in the development of SLE

The female versus male (F/M) ratio of SLE patients is reported to range between 7:1 and 20:1 (3, 19). In a representative mouse model of SLE, NZB x NZW F1 (B/WF1) mice, the disease was observed to be more severe in female than in male mice (20). Sex hormones, especially oestrogen, may well be involved in the reason for gender

bias of SLE although it appears to be complicated. For instance, the onset and/or activity of SLE are influenced by the menstrual cycle or pregnancy (21). In general, the disease activities of SLE tend to be exacerbated during the premenstrual period. A flare-up of SLE commonly occurs during early pregnancy, as well as in the puerperium (22-24). These phenomena suggest a close relationship between increasing concentrations of plasma oestrogen and exacerbation of SLE (4, 7). In contrast, the increase of endogenous steroids derived from the placenta during pregnancy may explain why the majority of women with SLE have a successful delivery and why improvement occasionally occurs in their clinical and laboratory manifestations (2).

Evidence regarding the role of sex hormones in the gender bias of the incidence of SLE is summarised in Table I. At physiological levels of oestrogen principally act as an enhancer of certain immune responses, while androgens and progesterone act as natural suppressors (1). In fact, serum immunoglobulin levels and the humoral immune response are higher in female mice and humans in comparison to males (25, 26). An exacerbation of the disease manifestations after the administration of oestrogen and an improvement after the administration of androgen have been observed in mouse models of SLE (such as B/W F1 and MRL/lpr mice) (18, 27-31). Certain hormone therapies, such as hormone replacement therapy (HRT) in postmenopausal women and stimulation of ovulation, can induce an exacerbation of the activity of SLE, while treatment with an exogenous androgen (dehydroepiandrosterone; DHEAS) results in modest amelioration of disease manifestations (32-35). *In vitro* experiments on mouse and human cells have revealed that oestrogens can induce the expansion of autoreactive B and T cells and magnify the B cell response to mitogens (6, 36-39). The cytokine profile in SLE is T helper (Th)-2 dominant (40). Interleukin (IL)-6 and IL-10 (Th-2 cytokines) are the most potent activators of B cells, thus promoting both the proliferation and immunoglobulin production (41, 42). In

Table I. Possible important roles of sex hormones in the development of SLE.

Findings	References
1) Gender differences of immune responses and SLE	
a) Higher incidence of SLE in females than males	3, 19
b) More severe disease activities in females than in male SLE model mice (B/WF1)	20
c) Higher production of immunoglobulins and stronger humoral immune responses in females than males (mice and human)	25, 26
2) Sex hormones and SLE <i>in vivo</i>	
a) Enhancing effects of oestrogens and protective effects of androgens on mice SLE models B/WF1 and MRL/lpr	20, 27, 28, 29
b) Oestrogen-induced B cell activation and autoantibody production in normal mice strains	6, 30, 31
c) Onset or exacerbation of SLE after hormone replacement therapy or repeated cycles of ovulation therapy	32, 33, 34
d) Modest clinical improvement of SLE by androgen (dehydroepiandrosterone;DHEAS) treatment	35
e) Flare-up of SLE with pregnancy and menstrual cycles	21, 22, 23, 24
3) Effects of oestrogens on autoimmune-related cellular immunity	
a) Enhancement of autoreactive T and B cells through resistance to apoptosis in oestrogen-treated mouse cells	31, 36, 37
b) Increase of immunoglobulin production or mitogen-mediated B cell responses by oestrogen in human cells	38, 39
4) Modification of cytokine production by sex hormones	
a) Increase of IL-6 and IL-10 production mice <i>in vitro</i> by oestrogen	7
b) Increase of IL-2 and INF-gamma (Th-1) and decrease of IL-1, IL-10, and TNF-alpha (Th-2) production mice <i>in vivo</i> by antioestrogen	45, 46
c) Increase of IL-6 by oestrogen during the menstrual cycle	47
d) Close relationship between IL-4 secreting cell numbers and oestrogen during the menstrual cycle, and close relationship between IFN-gamma secreting cell numbers and DHEAS in men or premenopausal women	7, 48
5) Effects of sex hormones on the transcription of endogenous genes	
a) Increased transcription of HERV in SLE and oestrogen-induced HERV transcription	8, 9, 10, 49, 52, 53

B/WF1 mice, the administration of IL-6 or IL-10 accelerates the development of SLE, while anti-IL-6 or anti-IL-10 delays its onset (43, 44). In general, oestrogens stimulate the production of Th-2 cytokines such as IL-4, IL-6, and IL-10, while anti-oestrogens promote the production of Th-1 cytokines such as IL-2 and interferon (IFN)-gamma (7, 45-48). Therefore, there are numerous reports regarding the stimulatory effects of oestrogen on the autoimmune-related immune responses including cytokines and antibody production, and the contrary effects of androgens. Most of these representative investigations regarding the basic/important effects of oestrogens on SLE-related immune responses *in vitro* and *in vivo* have been performed in the past decade.

Several reports have indicated the possible important roles of HERV in the pathogenesis of SLE (8-10). The transcription of HERV genes such as HERV clone4-1 in SLE patients is increased in comparison to normal controls and translation of the HERV is also promoted by an inactivation of stop codons of SLE patients, and the resultant production of HERV proteins is observed in peripheral blood mononuclear cells (PBMC) of SLE, but not normal controls (9, 49). These HERV proteins seem to be related to autoantibody production, through molecular mimicry between HERV proteins and autoantigens such as RNP antigens (10). Furthermore, HERV proteins themselves are reported to induce SLE-like immune abnormalities *in vitro* (50). Normally epigenetic

Table II. Expression of oestrogen receptors.

Estrogen receptor	Relative expression of ERA and ERb ¹				Expression in SLE compared to normal controls	Correlation to SLEDAI or PSL dose in SLE
	CD4 ⁺ T cell	CD8 ⁺ T cell	B cell	Monocyte		
Alpha (ERa)	High	Low	Low	Low	High ²	No correlation
Beta (ERb)	Low	Low	High	Low	Low ³	Inverse correlation ⁴

¹These data were obtained from normal individuals by qRT-PCR.

²This may be associated with hyper-responsiveness to oestrogens and contribute gender biases of SLE.

^{3,4}These findings (especially on B cells) may be influenced by therapies of PSL. See manuscript.

Table III. Process of gene selection.

Category	Numbers of detected genes
Detected genes in PBMC using the DNA microarray system ¹	26,852
↓	
Genes showing strong change in some sampling points in only females, but not males	4,245
↓	
Among them, known genes showing similar fluctuation pattern in each sampling point in females	13
↓	
Statistical significant difference observed between normal persons and SLE patients in the active stage ²	6
↓	
Interesting genes in their function; TNFRSF14, SIRPG	2

¹PBMC samples from three normal females were collected once a week during a month with a normal menstrual cycle. At the same sampling points, samples were also collected from three healthy males as controls. Total RNA were isolated from the PBMC and applied for DNA microarray analysis (12).

²Patients samples were obtained from five females and two males with SLE.

mechanisms (heritable changes in gene expression that occur without a change in DNA sequence) such as methylation of DNA, which is regulated by DNA methyltransferase (DNMT)-1, contribute to the inhibition of transcription of HERV genes. Levels of this DNMT-1 in PBMC of SLE patients are lower than those of normal controls and this is related to the hyper-transcription of HERV in patients with SLE (49, 51). In addition, oestrogens are known to promote the translation of HERV in certain human cell lines (52, 53). Therefore, oestrogens may promote the production of HERV proteins as autoantigens although further precise investigations (*e.g.* relationship between DNMT-1 and oestrogens) are required to elucidate this mechanism.

Expression of oestrogen receptors in SLE patients

As described above, oestrogens appear to strongly contribute to the develop-

ment of SLE, so it is interesting to think about the quantitative/qualitative status of oestrogen receptors in SLE patients. Two types of oestrogen receptor (oestrogen receptor alpha; ERa, and beta; ERb) are expressed on T cells, B cells, and monocytes (54, 55). Quantitative analyses of ERs messenger RNA (mRNA) expression in normal individuals by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) revealed that CD4⁺ T cells express relatively high levels of ERa mRNA in comparison to ERb mRNA, whereas B cells express high levels of ERb mRNA and low levels of ERa mRNA, in addition, CD8⁺ T cells and monocytes express comparable low levels of both ERs (56, 57). These data appear to suggest that CD4⁺ T cells will primarily show responsiveness to oestrogen mediated via the ERa versus the ERb for B cells, although the precise functional differences between the alpha and beta form of the

ERs still remain unclear (58). Previous studies have suggested the existence of ERa gene polymorphism which may be associated with the disease manifestations of SLE (59, 60), although PBMC of SLE patients express wild-type ERa and ERb like those of normal individuals (61). qRT-PCR methods also revealed that quantitative levels of ERa mRNA in SLE patients (mainly expressed on CD4⁺ T cells) are higher than those of normal volunteers, while the expression of ERb mRNA is decreased in comparison to PBMC from normal controls (11). Because significant inverse correlations were observed between ERb mRNA and SLE disease activity index (SLEDAI) or the dose of prednisolone (PSL), the latter finding (especially on B cells) may be influenced by therapies of PSL. In contrast, there is no significant relationship between ERa mRNA and SLEDAI or the dose of PSL (11). These findings are summarised in Table II. The issue of the number or affinity of ERs in SLE patients is still controversial probably due to differences between experimental systems and methods among the reported studies (62). However, it seems that quantitative and/or qualitative abnormalities of ERs (especially ERa on CD4⁺ T cells) in SLE patients are associated with hyper-responsiveness to oestrogens and contribute the development of gender biases on the incidence of SLE.

Attenuation mechanism of oestrogen-mediated immune enhancement effects in females and its destruction in SLE

There is a possibility that the inhibitory mechanisms for stimulatory effects of oestrogens on immune responses exist in healthy females (but not males) thus helping to maintain the homeostasis of biological immune systems, and such mechanisms work insufficiently in the patients with SLE, as a result, SLE patients are more easily affected by oestrogen-mediated immune effects. In fact, as described above, the onset and/or disease activities of SLE are influenced by pregnancy and the menstrual cycle (19), furthermore, an increase of CD4⁺CD25⁺ regulatory T cells (Treg) numbers (which is thought to be asso-

ciated with the maintenance of immune tolerances) has been reported with menstrual cycles in healthy females and this phenomenon may contribute to the escape from oestrogen-mediated immune enhancement effects (63, 64). In order to confirm this hypothesis, fluctuations of gene expression in PBMC were examined during the menstrual cycle in normal females, and compared with gene expression in patients with SLE, using DNA microarray or qRT-PCR methods (12). The process of gene selection in that experiment is summarised in Table III (12). Among 26,852 genes detected in PBMC using by the DNA microarray system, there were thirteen known genes which showed significant quantitative changes during the menstrual cycles of females, but not in males. Among these genes, there were statistical quantitative differences between normal controls and SLE patients in six genes. Among these six genes, one gene, the tumour necrosis factor receptor superfamily, member 14 (TNFRSF14) (synonym: herpes virus entry mediator; HVEM) seem to be the most compatible with the above described hypothesis. TNFRSF14 is a ligand of B and T lymphocyte attenuator (BTLA) (65, 66), and is located on chromosome (1p36), thus suggesting a linkage with SLE based on a genome-wide search for susceptibility genes in SLE (67). BTLA provides an inhibitory signal to B and T cell activations and TNFRSF14-BTLA interaction is an important pathway regulating lymphocyte activation and/or homeostasis in the immune system (65, 66). The expression of TNFRSF14 mRNA on PBMC in normal females is increased with increased serum oestrogen levels during the menstrual cycle and the resultant attenuation of immune systems occurs via TNFRSF14-BTLA pathways to avoid an immune enhancement by oestrogens. In SLE patients, the expression of TNFRSF14 mRNA during the menstrual cycle is significantly lower in comparison to normal females but this attenuation mechanism possesses an insufficient action and, as a result, the patients with appears to be strongly influenced by the oestrogen-mediated immune enhancement effects. Some interesting genes, such as a signal regulatory

protein, gamma (SIRPG; a receptor of CD47 and the SIRPG-CD47 interaction is related to T cell activation) (68) were observed apart from TNFRSF14 among these final six genes (Table III). However, the failure of the TNFRSF14-BTLA systems in the patients with SLE during the menstrual cycle seems to be an interesting and important phenomenon in the development of gender bias in this disease (12).

Conclusion

Numerous investigations have addressed the gender bias mechanisms of SLE. In the majorities of those studies, sex hormones, such as oestrogens and pregnancy/menstrual cycles, were suggested to play an important role in the development of the onset/disease activities of SLE. Higher susceptibilities to the immune enhancement effects of oestrogens in the patients with SLE seem to be important in their gender difference, and these may be due to quantitative/qualitative abnormalities of their ERs and occur in association with an insufficiency of suppressive mechanisms such as the TNFRSF14-BTLA pathways for these oestrogen effects. Generally, a failure of these immune attenuation systems (including Treg) may be related to the development of autoimmune diseases such as SLE and rheumatoid arthritis (RA). Furthermore, recently developed biological technologies for comprehensive gene analyses such as DNA microarray, as well as widely applied protein analyses using mass spectrometry (69), appear to be very useful for pathogenic studies of these autoimmune diseases induced by complicated factors, including gender research for SLE. These new methodologies should thus result in further useful and important observations in autoimmune disorders, while also opening up new possibilities in the treatment and diagnosis of these disorders.

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