Gender influences SLE-immune cells, genetics, experimental models and lupus patients

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Systemic lupus erythematosus (SLE) is the prototypic multi- system autoimmune disease characterised by altered immune functions leading to the development of multiple autoantibodies. The disease predominantly affects females of reproductive age. The pathogenesis of SLE has not been completely elucidated, but it seems to encompass multiple factors such as infections, ultraviolet exposure, endogenous and exogenous hormones, and genetic characteristics (1-4).

In general, females exhibit stronger immune responses to a variety of antigens than males. The higher immune reactivity in females translates into faster responses to infectious agents, but also into a higher preponderance for autoimmunity (5). In the past decade, mounting evidence suggests that gender bias in murine experimental lupus models is influenced by sex hormones. Immunomodulation by the female sex hormones oestrogen and prolactin accelerates the onset of disease and leads to premature death in the spontaneous lupus model of NZB/W F1 mice. Also, hormonal therapy that induce persistently increased serum levels of estrogen or prolactin can provoke a lupus-like phenotype in mice that are not predisposed to the disease (6). Murine models provide information on the effect of sex hormones on B cell survival, maturation, activation, and antibody production. Both oestrogen and prolactin impair normal deletion mechanisms for removal of autoreactive B cells and accelerate their maturation and ability to secrete self-reactive antibodies (7, 8).

The effects of oestrogen effects are mediated *via* oestrogen receptors (ERs) alpha and beta (ER α and β) that are expressed on a variety of immune cells. Genetic deficiency of ER α in lupus-prone mice results in significantly decreased disease activity and prolonged survival, whereas ER β deficiency has minimal to no effect. Thus, modulation of ER α function appears to be a potential target for therapy in autoimmunity (9).

Despite the extensive studies of the interplay between oestrogen and autoimmune processes, such mechanisms have yet to be elucidated. In general, oestrogen skews the immune system towards the Th2 axis and promotes antibody secretion with potential implications for autoimmune responses. In addition, in B lymphocytes, functional binding sites for ERs are identified in the promoter of the gene encoding the enzyme required for somatic hypermutation and class-switching known as activation-induced deaminase (AID). These observations support the notion that oestrogen can affect antibody production and affinity-maturation and further suggests a potential mechanism for oestrogen-mediated production of pathogenic high-affinity autoantibodies (10).

The interferon (IFN-α) signature of SLE, described by multiple investigators over the past several years, seems to be affected by oestrogen. IFN-α is expressed by the placenta and is postulated to upregulate this cytokine in females leading to increased reproductive fitness and simultaneously increased susceptibility to SLE (11). Interferon-inducible Ifi200-family genes found in mouse and humans cluster between serum amyloid P component and alpha-spectrin genes on chromosome 1 and in the human 1q23. *In vivo*, estradiol treatment of orchiectemised male lupus-prone mice leads to an increase in the level of Ifi202 mRNA in splenic cells (12). Gene profiling reveals differential expression of oestrogen responsive genes between SLE and normal T cells. Signal transduction pathways are altered by estradiol in lupus and the IFN-αl pathway is upregulated in response to estradiol in SLE T cells (13).

Oestrogen affects not only B and T lymphocytes, but also dendritic cells (DCs). In a lupus prone murine model, oestrogen stimulated activity of immature DCs, but suppressed mature DCs. In addition, oestrogen decreased the release of IL-6, IL-10, IL-12, and TNF-alpha of DCs and also changed the expression of ER α in DCs (14).

The hormonal effects on the immune system are also influenced by genetic factors. For example, the lupus susceptibility locus *Sle3* along with a transgene that encodes the heavy chain of a pathogenic anti-DNA antibody together, but not separately, allow for prolactin to induce the development of lupus in mice that are not otherwise predisposed to the disease (15).

Recently, gender associated genetic factors have shed light to their added influence on the process of lupus development. Both the number of X-chromosomes and genetic variants on the X-chromosome are related to the risk for development of SLE. Two functional X chromosomes, either by sex or by translocation or duplication, appear to confer a greater risk of lupus than one X-chromosome (11). In addition, the Yaa mutation on the Y chromosome of male BXSB lupus prone mice contains a duplicate gene that encodes for the X-chromosome harbouring TLR7 causing alteration in the immune response towards hyperactivity (7).

The evidence for a sex hormone influence is based on the facts that lupus is a predominantly female disease characterised by a peak incidence during the reproductive age and an amelioration of lupus activity in the post-menopausal years, as well as by induction or worsening during pregnancy. SLE patients may have menstrual abnormalities reflecting altered ovarian function (16). Medications that cause ovarian failure may ameliorate SLE activity whereas high-oestrogen containing contraceptives, or increased oestrogen exposure during *in vitro* fertilisation can worsen lupus. However, recent evidence points to the possible non-deterrent effect of modern oral contraceptives partly due to the low doses of oestrogen compared to those of decades ago. Hormone replacement therapy (HRT) in the post-menopausal woman does not lead to major flares. Substantial data is lacking on the possible danger of *in vitro* fertilisation in lupus patients (17), but large quantities of exogenous estrogen utilised for transgender sex reassignment surgery may lead to the development of lupus (18). Industrial estrogens as found in plastics and pesticides may further increase the risk for lupus. Alternative medicine substances sold over the counter such as plant or fungal sources of oestrogen (phytoestrogen) may also have effects on the immune system (19). Chronic exposure to xenoestrogens of the pesticide group (DDT and TCDD) appeared to accelerate the development of albuminuria in a spontaneous lupus mouse model (20). Further clinical and pre-clinical trials are necessary to assess the environmental potential of minor, but prolonged exposure to sex hormones. Progesterone, previously believed to be harmless was shown to play a role in lupus nephritis in a mouse model (21). Androgens have a protective effect. Female individuals have lower androgen levels. Males with Klinefelter syndrome possess lower male sex hormones and a few cases of lupus have been reported in this group. DHEA, a synthetic mild testosterone- like drug, or its metabolite, 7-hydroxy androstene steroid, may have some beneficial effect for mild SLE (17, 22).

In this issue, Sekigawa *et al*. (23) review the hormonal mechanisms of gender differences in SLE in experimental models and in humans. The review explores some recently reported facets of lupus and provides novel aspects in this research area. The authors discuss the possible role of oestrogen in increased transcription of the genes encoding human endogenous retroviruses (HERV) and thereby in elevated levels of HERV proteins in the peripheral blood mononuclear cells (PBMCs) of lupus patients. This observation indicates a new mechanism for oestrogen-mediated breakdown of immune tolerance because HERV proteins are related to autoantigens such as RNP and can promote the production of autoantibodies. Sekigawa's review also discusses the controversies surrounding the number and affinity of ERs in SLE, and stresses that abnormalities in ERα in lupus $CD4^+$ T cells may lead to enhanced responsiveness to oestrogen and thus contribute to the female preponderance of SLE. In addition, the review addresses the insufficiency of suppressive mechanisms for oestrogen-mediated immune hyper-reactivity in SLE. DNA microarrays and qRT-PCR experiments utilised to compare gene expression in PBMCs from SLE and healthy women during menstrual cycle identified differences in 6 genes (24). From these 6 genes, the TNF receptor superfamily member 14 (TNFRSF14), also known as herpes virus entry mediator (HVEM), seems to be of highest significance. TNFRSF14 serves as a ligand for B and T lymphocyte attenuator (BTLA) and the TNFRSF14-BTLA interactions regulate lymphocyte activation. During menstrual cycle, the expression of TNFRSF14 rises along with the increases in serum oestrogen levels and thereby attenuates immune responses by the TNFRSF14-BTLA pathway. In SLE patients, the TNFRSF14 expression does not follow elevated oestrogen levels adequately which results in a higher TNFRSF14-BTLA pathway activity in response to oestrogen than in healthy women.

With all this pondered, the higher susceptibility to the immune effects of oestrogens in SLE patients seems to play an important role in the gender bias seen in lupus. Newly developed methodologies for gene and protein analysis such as the microarray technology and mass spectrometry, respectively, may provide very useful novel tools for studying complex autoimmune diseases such as SLE. These technologies are expected to open new horizons for identifying novel diagnostic and therapeutic targets for autoimmune disorders.

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