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Editorial

Why Is Systemic Lupus Erythematosus More Common in Women?

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1. Introduction

Compared to males, females usually have stronger immune responses and greater susceptibility to autoimmune diseases such as systemic lupus erythematosus (SLE). SLE is a heterogeneous multifactorial autoimmune disease whose pathogenesis involves both genetic and environmental factors. Key mechanisms in the loss of immune tolerance and consequent tissue damage include impaired clearance of immune complexes and apoptotic bodies, production of neutrophil extracellular traps and antibodies to DNA or ribonucleoproteins (RNPs), and sustained production of type 1 interferons (IFNs) [1,2]. The marked predominance of females among patients with SLE has long suggested a pathogenic role for female hormones. Convincing evidence has been obtained that estrogens directly influence the survival, development, or function of immune cells involved in SLE, such as B cells [3–6] and plasmacytoid dendritic cells (pDCs), which are the main sources of type 1 IFNs [7,8,9,10]. However, the female predominance also exists in prepubertal pediatric patients with SLE, indicating that sex hormones are not the only factor involved [11]. Furthermore, several studies have demonstrated that susceptibility to SLE in males with Klinefelter syndrome (47, XXY) is similar to that in females, suggesting a role for X-linked genetic factors [12,13]. The X chromosome carries many genes that are directly or indirectly involved in immunity. Among them, *TLR7*, which is located on the short arm of the X chromosome and encodes toll-like receptor 7, is a prime candidate as an SLE susceptibility gene. *TLR7* is indeed expressed by human pDCs, monocytes, and B cells. TLR7 recognizes single-strand RNA and is crucially involved in B-cell responses in SLE including the production of anti-RNP antibodies, as well as the production of type 1 IFNs by pDCs [2]. Many studies in experimental SLE models have consistently found evidence of a pivotal role for *TLR7* in the development of SLE. TLR7 overexpression suffices to induce a lupus-like disease whereas, on the other hand, TLR7 knockout inhibits the development of lupus in murine models [14–16].

This editorial discusses the available data supporting a role for sex hormones, notably estrogens, in the female SLE bias. In addition, , and recent findings from our laboratory demonstrating that *TLR7* escapes from X-chromosome inactivation (XCI) in immune cells from females or in males with Klinefelter syndrome and that biallelic *TLR7* expression enhances B-cell functional responses [17]. Taken together, these data suggest that estrogens and escape of *TLR7* from XCI may contribute independently to the greater propensity of females to produce anti-RNP antibodies and to develop SLE.

2. Gender bias in systemic lupus erythematosus (SLE): role for estrogens

SLE is characterized by a strong gender bias, with females being affected about 9 times more often than males [18,19]. The onset of SLE usually occurs in women of childbearing age and is less common after menopause, suggesting that estrogens may constitute a susceptibility factor for the disease [18]. In keeping with this possibility, pregnancy or exposure to exogenous estrogens was shown in several studies to exacerbate the disease [20]. The same gender bias exists in most murine SLE models. An exception, however, is the BXSB model, in which males carry an autoimmune accelerator mutation on the Y chromosome (Yaa) that accelerates the progression of a spontaneous lupus-like disease. This unusual susceptibility of males has been ascribed to translocation of the short arm of the X chromosome onto the Y chromosome, so that males have an additional copy of the *TLR7* gene [15]. In murine models of SLE chiefly affecting females, exogenous 17β-estradiol shortened the time to disease onset and increased disease severity, whereas castration had a protective effect [21,22]. Estrogens are produced by the ovaries, and their functions are mediated by two types of estrogen receptor (ER), ERα and ERβ. ERs participate in numerous physiological processes in mammals and are involved in the development of many diseases [23]. In lupus-prone mice, ERα deficiency attenuated the severity of the disease, suggesting direct effects of estrogens on immune cells [24]. B cells and pDCs are among the cell populations believed to be directly affected by estrogens. By enhancing the expression of CD22 and SHP-1 by B cells, estrogens decreased the signaling threshold of the B-cell receptor and allowed autoreactive B-cell survival via positive regulation of the B-cell lymphoma 2 protein and of B-cell activating factor [3,4]. Estrogens may also promote somatic hypermutation by increasing the expression of the enzyme activation-induced cytidine deaminase [5]. Further support for an in vivo effect of estrogens on B cells in patients with SLE comes from a recent study demonstrating that *ERα* deletion in B cells resulted in decreased disease severity in (NZBxNZW)F1 mice [6]. In addition to B cells, pDCs have been shown in many studies

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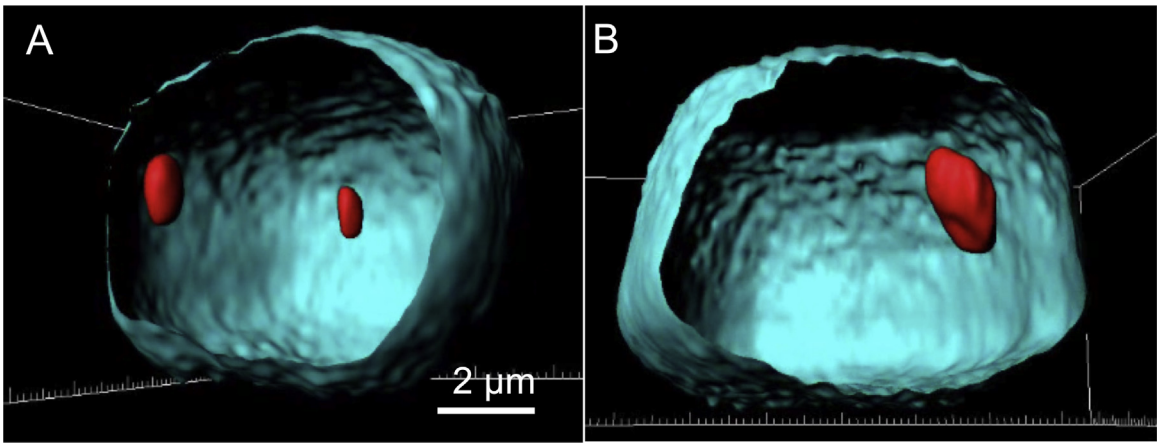

Fig. 1. RNA-FISH visualization of primary TLR7 transcripts (in red) in the B cells of a patient with Klinefelter syndrome (47, XXY). The B cells were stimulated using TLR7 ligands and interferon beta for 5 days before in situ hybridization using probes specific for primary *TLR7* transcripts, as reported previously [17]. (A) Biallelic cells with two red foci indicating the primary *TLR7* transcripts located on the two X chromosomes. (B) Monoallelic cell with a single red focus.

to be strongly regulated by estrogens [25]. These cells are the main population of type 1 IFN-producing immune cells after activation via their TLR7 or TLR9 by nucleic acids from pathogens [26]. Another stimulus responsible for IFN-β production by pDCs is inappropriate activation of these TLRs by self nucleic acids bound in complexes to autoantibodies, which contributes to the pathogenesis of SLE [2,27]. Activation of autoreactive B cells and the production of autoantibodies that produce pathogenic immune complexes promote the release of type 1 IFNs by pDCs, which in turn results in monocyte maturation to dendritic cells, activation of autoreactive T cells, B-cell maturation, and autoantibody production by plasma cells [2,27]. Interestingly, a marked gender bias exists in type 1 IFN production by human pDCs, with pDCs from females releasing larger amounts of type 1 IFN in response to stimulation by TLR7 [25]. We recently investigated the molecular mechanisms underlying this gender bias [25]. By using mice whose dendritic cell lineage was deficient in REα, we demonstrated an intrinsic role for estrogens in IFN-α production by pDCs from females [7,10]. In a humanized mouse model, we not only confirmed that estrogens affected the functions of human pDCs, but also noted unexpectedly differences depending on the gender of the human stem-cell donors [9]. After stimulation by TLR7, the proportion of IFN-α producing cells was higher among female (XX) than male (XY) pDCs, in both male and female recipient mice [9]. Given that the *TLR7* gene is on the X chromosome, we hypothesized that it might escape from X-inactivation in certain immune cells and must therefore be expressed as two copies to be effective, so that biallelic cells would have a stronger functional response to TLR7 ligands compared to monoallelic cells.

3. Chromosome X dosage and susceptibility to systemic lupus erythematosus (SLE)

The X chromosome carries over a thousand genes including microRNAs and many genes encoding proteins known to directly or indirectly control the immune responses [19]. The genes encoding two endosomal TLRs specific of single-strand RNA, *TLR7* and *TLR8*, are located on a non-pseudoautosomal region of the short arm of chromosome X. As pointed out above, the predominance of females among SLE patients and the fact that males with Klinefelter syndrome, who have at least two X chromosomes, are as susceptible as females to SLE and Sjögren's syndrome [12,13] strongly suggest that a higher chromosome X dosage is per se a risk factor for SLE.

The effect of the X chromosome complement on susceptibility to autoimmune disease was studied in mice whose testes-determining *Sry* gene was deleted from the Y chromosome (Y-), producing XY- mice with ovaries [28]. *Sry* was then inserted as a transgene on an autosomal region (Sry+Tg). This model allows an assessment of X chromosome effects that are independent from hormones. Susceptibility to induced [28] or spontaneous [29] lupus was greater in the XX females than in the XY- females and in the Sry+Tg XX males than in the Sry+Tg XY- males, suggesting that susceptibility to lupus may be influenced by the X chromosome dosage. Genetic mechanisms that may explain the effect of chromosome X on the susceptibility to autoimmune diseases include biallelic *TLR7* expression due to escape from X-inactivation, which we demonstrated recently [17].

4. TLR7 escapes from X-inactivation

In female mammals, one of the two X chromosomes is randomly inactivated to ensure that the amount of X-chromosome gene products is similar in females and males. XCI in females is incomplete, with 15% to 23% of X-linked genes remaining active [30,31]. This fact suggests the possibility that the greater susceptibility to SLE in females may be related to overexpression of X-linked genes, such as *TLR7*, due to escape from XCI in certain immune cells [19].

We recently investigated escape from XCI in primary populations of immune cells from females and the functional consequences of biallelic *TLR7* expression, notably on B-cell responses [17]. We used reverse transcription polymerase chain reaction technology on single cells to assess monoallelic and biallelic *TLR7* expression in women heterozygous for *TLR7* exonic single nucleotide polymorphisms. The results established that *TLR7* escaped from X-inactivation in pDCs, B cells, and monocytes from females. In addition to the cells exhibiting the expected monoallelic expression of the gene on the paternal or maternal X chromosome, all the study participants had biallelic *TLR7* expression, which was found in 7% to 45% of cells depending on the individual. To achieve in situ visualization of primary *TLR7* transcripts, we developed an RNA-FISH technique in which the inactive X chromosome is identified via detection of the long noncoding RNA X-inactive specific transcript (XIST). Using this method, we documented the presence of primary *TLR7* transcripts on the inactive X chromosome of B cells, monocytes, and pDCs not only in women, but also in males with Klinefelter syndrome (47, XXY). Fig. 1 shows a 3D reconstruction of two B-cell nuclei from a 47, XXY male after RNA-FISH labeling of primary *TLR7* transcripts, one from a biallelic cell (A) and the other from a monoallelic cell (B), as reported previously [17]. At the single-cell level, biallelic *TLR7* expression was associated with a selective increase in *TLR7* transcripts in naive B cells. Using a

monoclonal antibody specific of the protein to assess leukocytes from healthy donors, we demonstrated TLR7 overexpression in the cells from females compared to males under basal conditions. Support for a causal link between biallelic *TLR7* status and function comes from our finding of better memory B cell differentiation to CD27 + plasmablasts upon *TLR7* engagement in *TLR7* biallelic B cells from women exhibiting biallelic *TLR7* expression. Similarly, naive B cells with biallelic *TLR7* expression exhibited enhanced differentiation to IgG + plasma cells in the presence of TLR7 ligands. Given the key role for TLR7-dependent signaling in B-cells in patients with lupus, these data suggest a major role for this mechanism in the greater susceptibility to SLE of females and of males with Klinefelter syndrome [17].

In conclusion, although sex hormones play a considerable role in the susceptibility to SLE, X-linked genetic factors also contribute to this gender bias. The long suspected contribution of escape of the *TLR7* gene from XCI in females and 47, XXY males has been now established. These recent findings link the presence of two X chromosomes to a better TLR7-dependent B-cell response [17]. This link may explain why females and males with Klinefelter syndrome are similarly susceptible to TLR7-dependent autoimmune diseases. The direct contribution of this mechanism to the pathogenesis of SLE now requires further investigation.

Disclosure of interest

The author declare that he have no competing interest.

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Jean-Charles Guéry [a,b,c,*]
[a] *Centre de Physiopathologie de Toulouse Purpan (CPTP), 31024 Toulouse Cedex 3, France*
[b] *Université de Toulouse, 31300 Toulouse, France*
[c] *Inserm, CNRS, UPS, 31300 Toulouse, France*

[*] Corresponding author: Centre de Physiopathologie de Toulouse Purpan, Centre Hospitalier Universitaire Purpan, place du Dr. Baylac, 31024 Toulouse Cedex 3, France
E-mail address: Jean-Charles.Guery@inserm.fr

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