Seminars in Immunopathology (2019) 41:153–164
https://doi.org/10.1007/s00281-018-0712-y

REVIEW

# Female predisposition to TLR7-driven autoimmunity: gene dosage and the escape from X chromosome inactivation

Mélanie Souyris[1] · José E. Mejía[1] · Julie Chaumeil[2] · Jean-Charles Guéry[1,3]

Received: 29 June 2018 / Accepted: 17 September 2018 / Published online: 1 October 2018


## Abstract

Women develop stronger immune responses than men, with positive effects on the resistance to viral or bacterial infections but magnifying also the susceptibility to autoimmune diseases like systemic lupus erythematosus (SLE). In SLE, the dosage of the endosomal Toll-like receptor 7 (TLR7) is crucial. Murine models have shown that TLR7 overexpression suffices to induce spontaneous lupus-like disease. Conversely, suppressing TLR7 in lupus-prone mice abolishes SLE development. TLR7 is encoded by a gene on the X chromosome gene, denoted *TLR7* in humans and *Tlr7* in the mouse, and expressed in plasmacytoid dendritic cells (pDC), monocytes/macrophages, and B cells. The receptor recognizes single-stranded RNA, and its engagement promotes B cell maturation and the production of pro-inflammatory cytokines and antibodies. In female mammals, each cell randomly inactivates one of its two X chromosomes to equalize gene dosage with XY males. However, 15 to 23% of X-linked human genes escape X chromosome inactivation so that both alleles can be expressed simultaneously. It has been hypothesized that biallelic expression of X-linked genes could occur in female immune cells, hence fostering harmful autoreactive and inflammatory responses. We review here the current knowledge of the role of TLR7 in SLE, and recent evidence demonstrating that *TLR7* escapes from X chromosome inactivation in pDCs, monocytes, and B lymphocytes from women and Klinefelter syndrome men. Female B cells where *TLR7* is thus biallelically expressed display higher TLR7-driven functional responses, connecting the presence of two X chromosomes with the enhanced immunity of women and their increased susceptibility to TLR7-dependent autoimmune syndromes.

**Keywords** Sexual dimorphism · Systemic lupus erythematosus · Toll-like receptor 7 · X chromosome dosage · X chromosome inactivation escape

## Introduction

The quality and strength of the immune response differ between women and men, resulting in sex-based differences in the prevalence, manifestations, and outcome of autoimmune and infectious diseases. While women are able to mount more vigorous immune responses to infections, they also suffer more from autoimmunity and subsequent inflammation-induced tissue damage. A growing body of data shows that biological pathways common to autoimmune and infectious diseases are under the control of sex-linked factors [1–3]. The role of sex hormones, estrogens and androgens, is being actively explored [4–6] and, more recently, interest has arisen also in the control of immunity directly by the number of sex chromosomes in the karyotype. An important question in the biology of sex chromosomes is balancing the gene dosage between male (XY) and female (XX) cells. Indeed, the X chromosome carries many more genes than the Y chromosome, so that most X-linked genes exist as two copies in female cells versus only one in male cells. Thus, in mammals, this gene dosage imbalance is offset by the inactivation of one of the two X chromosomes in female cells. The impact on immunity of gene expression that evades this X chromosome

This article is a contribution to the special issue on Sex differences in immunity – Guest Editors: Hanna Lotter and Marcus Altfeld

✉ Jean-Charles Guéry
Jean-Charles.Guery@inserm.fr

[1] Centre de Physiopathologie de Toulouse Purpan (CPTP), Université de Toulouse, INSERM, CNRS, Université Paul Sabatier, 31300 Toulouse, France

[2] Institut Cochin, INSERM U1016, CNRS UMR8104, Université Paris-Descartes, 75014 Paris, France

[3] INSERM UMR1043, Centre Hospitalier Universitaire Purpan, Place du Dr. Baylac, 31024 Toulouse Cedex 3, France

inactivation (XCI) is emerging as a promising field of study. We highlight recent relevant data on a key immune receptor encoded by an X-linked locus, Toll-like receptor 7 (TLR7), and its link to the pathophysiology of the autoimmune disorder, systemic lupus erythematosus (SLE).

## X chromosome inactivation in lymphoid cells

In mammals, female cells carry an XX pair of sex chromosomes, whereas males have a Y chromosome and a single X. Men with Klinefelter syndrome, however, carry at least one additional X chromosome (47,XXY). Dosage compensation between XY males and XX females for X-linked gene products occurs via random epigenetic silencing one of the two female X chromosomes. This process, XCI, begins at the four-cell stage during embryonic development and is inherited through somatic cell divisions over life. XCI is dependent on epigenetic features including the transcription of the X-inactivation specific transcript (XIST), a long non-coding RNA (lncRNA) encoded by an X-linked gene, chromatin modifications, and nuclear organization [7]. At the onset of XCI, the *XIST* allele on the future inactive X chromosome (Xi) is upregulated and XIST RNA accumulates along this chromosome in *cis*. This event initiates the silencing of X-linked genes as well as the recruitment of the polycomb repressive complex 2 (PRC2) and the acquisition of other epigenetic features involved in the stable maintenance of the inactive state. In particular, the histone methyltransferase activity of PRC2 catalyzes the deposition of the repressive histone mark, the methylation of lysine 27 on histone H3 (H3K27me3), which is known to form transcriptionally inactive heterochromatin domains [7]. The chain of early events in XCI has been studied by RNA fluorescence in situ hybridization (RNA FISH) [8]. The XIST-coated chromatin can be detected in interphase nuclei as a domain covering ~ 70% of the X chromosome territory (often called the XIST cloud or XIST domain), whereas the *XIST* allele on the other X chromosome is progressively silenced. XIST RNA deposition is followed by progressive transcriptional silencing of X-linked genes, based on the disappearance of primary transcript RNA FISH signals from the XIST domain [8]. Thus, the Xi and the active X chromosome (Xa) harbor distinct epigenetic modifications and gene expression patterns. However, consistent transcription of both alleles persists in a small minority of X-linked genes with functional homologs on the Y chromosome, clustered in the so-called pseudoautosomal regions, PAR1 and PAR2 (Fig. 1).

XIST expression is supposed to be maintained in differentiated cells although little is known about regulation of this lncRNA in immune cells. Loss of XIST clouds, and H3K27me3 depletion, has been observed during B and T lymphocyte development in mice [11, 15, 16]. RNA FISH-

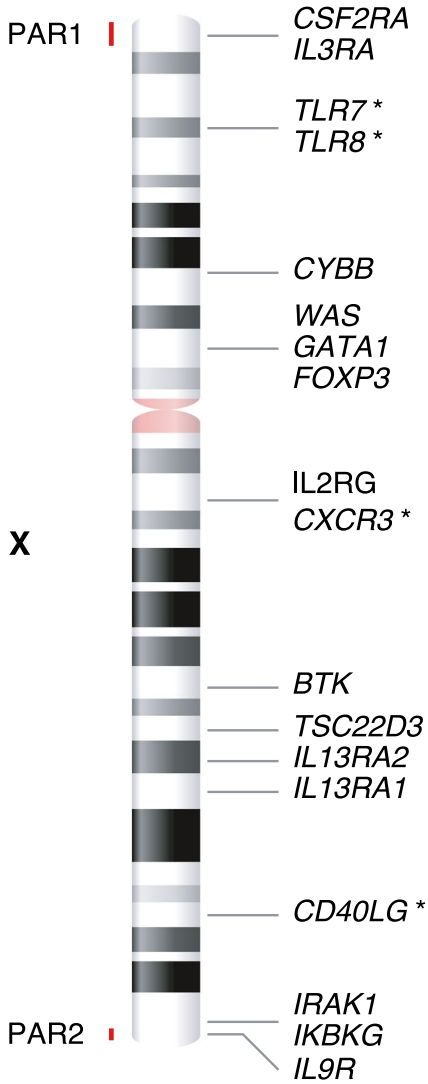


**Fig. 1** Selected genes on the X chromosome with a known potential to influence immunity. The X chromosome contains some 1100 genes, of which 60–100 have been reported to influence directly or indirectly innate and adaptive immunity, see references [1, 9]. *TLR7* and *TLR8* are located on the short arm of the X chromosome within a region reported to escape XCI [10]. Asterisks denote genes reported or suspected to escape XCI, according to references [11–14]

detectable XIST domains nearly disappeared in immature double-positive T and pre-B cells, while about 50% of mature $CD8^+$ single-positive T and B cells regained a XIST domain [15]. Subsequent studies by Anguera and colleagues found that, by contrast, both human and mouse naïve $CD4^+$ and $CD8^+$ T cells and B lymphocytes still lacked XIST RNA and the heterochromatic histone modifications associated with the Xi at the steady state [11]. This loss of XIST staining in resting lymphocytes was transient, and XIST RNA relocalization to the Xi could be observed during the transition from quiescence to activation [11, 16]. In particular, it was shown that, upon B cell activation, the XIST RNA localization to the Xi was restored in a dynamic two-step process involving the transcription factor YY1, which is known to

regulate processes during V(D)J recombination [16]. These results suggest that, at the steady state, naïve B cells are predisposed to X-linked gene reactivation [16], and hence to the possibility of non-compensation of X-linked gene dosage between female and male cells. Similar results were found in naïve and activated T cells from humans and mice, suggesting that the Xi is predisposed to become partially reactivated in mammalian female lymphocytes at large [11]. Such loss of heterochromatin features of the Xi in lymphocytes is in agreement with a recent study profiling the open regions of chromatin in resting human $CD4^+$ T cells [17]. Using transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) to map the regions of open chromatin in female and male human $CD4^+$ T cells, particular regions of the X chromosome were observed to produce twice higher signals in female than in male cells, which were thought to represent genes escaping XCI [17].

## Role of TLR7 in SLE

A notable shared mechanism in autoimmunity and the fight against infections is pattern recognition by innate immune receptors such as TLRs. The innate response initiated by TLRs is an essential line of defense against many pathogens; however, TLRs can also respond to endogenous ligands, potentially leading to autoimmunity or inflammation if not properly controlled [18]. This is particularly clear for TLR7, a single-stranded RNA (ssRNA) receptor encoded by an X-linked gene.

A key role for TLR7 has been established in SLE, a prototypic autoimmune disease with strong female bias. TLR7 recognition of endogenous RNA results in the production of type I interferon (IFN) and antiribonucleoprotein (RNP) autoantibodies [18, 19]. In mice, an increased copy number of the *Tlr7* gene leads to a lupus-like syndrome [20–22], whereas *Tlr7*-deficient mice of a lupus-prone genetic background are significantly protected from autoimmunity [19]. Consequently, it has been hypothesized that gene-dose effects due to escape from XCI contribute to the sex bias in SLE susceptibility [1].

SLE is a chronic disorder that is clinically heterogeneous in its manifestations and course. Life-threatening inflammation and damage may involve many organs and tissues, including the kidney, skin, joints, central nervous system, and serosae. SLE is characterized by high-affinity autoantibodies against macromolecular complexes with a nucleic acid component: RNA associated with the U1 small nuclear RNP or the Ro60 autoantigen, and double-stranded DNA (dsDNA) [18, 23]. SLE patients show elevated levels of circulating IFN-α, which is correlated with the presence of autoantibodies to RNA binding protein [24], and overexpression of IFN-α-regulated genes in blood cells, suggesting a central role for type I IFN in disease pathogenesis [25–29]. Plasmacytoid dendritic cells (pDCs) constitute the main blood cell population producing type I IFN upon activation via TLR7 or TLR9 by pathogen-derived nucleic acids [30]. IFN-α production by pDCs can also result from inappropriate activation of these TLRs by self-nucleic acids complexed with autoreactive antibodies, thereby contributing to the pathogenesis of SLE [31–33]. A disease paradigm has thus emerged in SLE, in which activation of autoreactive B cells and production of autoantibodies forming pathogenic immune complexes promote IFN-α/β release by pDCs. Type I IFNs contribute to sustain inflammatory responses through their capacity to drive monocyte maturation into dendritic cells, enabling activation of autoreactive $CD4^+$ T cells which can provide help to differentiate $CD8^+$ T cells and B cells into autoreactive effectors [34]. pDC-driven type I IFNs also promote the differentiation of activated B cells into plasmablasts [34], and combined activation of type I IFN and TLR signaling in human B cells expands $IgM^+$ memory cells and plasma cell (PC) lineage-committed $CD27^{hi}$ B cells [35].

Moreover, nucleic acid-containing apoptotic particles promote activation of autoreactive B cells via dual B cell receptor- and TLR-mediated signals, which accounts for the prominence of antinuclear antibodies (ANAs) in autoimmunity [18]. The Myd88-dependent, endosomal receptors TLR7 and TLR9 are critical in this context, with TLR7 required for the generation of antibodies to RNA and RNA-associated proteins [36], whereas TLR9 activation promotes production of antibodies to dsDNA and chromatin [19, 37, 38].

Abnormal expression of the *Tlr7* gene has been linked to SLE pathogenesis in the lupus-prone BXSB strain of mice. Male BXSB animals carrying a supernumerary copy of the *Tlr7* gene contributed by the Y-linked autoimmune accelerator (Yaa) locus displayed a twofold increase in *Tlr7* expression and spontaneously developed lupus-like disease, associated with glomerulonephritis, and the production of high titers of autoantibodies directed against cardiolipin and the U1 small nuclear RNP (Sm/RNP). Female mice, with only one active copy of *Tlr7*, developed a milder disease [20, 21]. The Yaa locus results from a segmental translocation of at least 16 X-linked genes to the Y chromosome, but the extra copy of *Tlr7* in Yaa-carrier males is the dominant genetic contributor to the Yaa phenotype [22, 39, 40]. Furthermore, four- to eightfold overexpression of *Tlr7* is sufficient for the spontaneous onset of SLE in non-autoimmune strains [22], and selective suppression of *Tlr7* expression in lupus-prone mice is, conversely, protective against the disease [41].

Several studies have examined the cellular requirements for spontaneous autoimmunity in mice with excessive *Tlr7* gene expression. *Tlr7* expression in both the innate and adaptive compartments was found to be necessary for SLE development, where TLR7-induced B cell mediated autoimmunity was dependent on T cell-derived signals [42–44]. TLR7 may

control SLE development at multiple levels. In B cells, TLR7 exerts a non-redundant role by promoting spontaneous germinal center (GC) and B cell plasmablast development in lupus-prone mice [44, 45]. Sera from SLE patients contain anti-Sm/RNP antibody complexes that can promote type I IFN production by pDCs through TLR7 activation [46]. Given the central role of pDCs in spontaneous SLE mouse models [47, 48], TLR7-dependent signaling that elicits type I IFN production by pDCs is a likely contributor to SLE development. Indeed, pDC-derived type 1 IFNs have been reported to prime neutrophils for NETosis, which is initiated by the uptake of Sm/RNP immune complexes, and subsequent engagement of TLR7 in neutrophils by the RNA component of these immune complexes [49, 50]. The DNA in the neutrophil extracellular traps formed by dying neutrophils associates with antimicrobial peptides or anti-DNA IgG, and goes on to engage TLR9 in the endosomal compartment of pDCs, leading to type I IFN release by these cells, which further fuels the autoinflammatory processes [51]. Circulating Sm/RNP-containing immune complexes have also been reported recently to be critical for ssRNA ligand-dependent TLR7 activation and upregulation of OX40 ligand by human monocytes, thereby contributing to human lupus pathogenesis by promoting T follicular helper (Tfh) cell development [52].

The non-redundant requirement for TLR7 expression by innate cells (pDCs, monocytes, or neutrophils) in TLR7-driven SLE pathogenesis remains to be formally established. By contrast, the critical role of cell-intrinsic expression of *Tlr7* in B cell responses and GC reactions has been well documented in a variety of pathophysiological contexts (including both SLE and viral infection). TLR signaling through Myd88 is necessary for B cell survival, differentiation, and proliferation, and it potentiates B cell migration to GC dark zones and polyclonal activation of memory B cells [35, 53–56]. Cell-intrinsic activation of TLR7 has been shown to directly contribute to the functional responses of B cells, in particular the production of antibodies against viral determinants [57–60] or the Sm/RNP autoantigen in SLE [36, 44, 45, 61, 62]. In BAC transgenic mice, moderate overexpression of *Tlr7* was sufficient to drive SLE when combined with carriage of the *Sle1* locus [62]. Restoring the normal *Tlr7* copy number specifically in B cells significantly decreased the titer of antibodies to RNA-protein complexes and protected from SLE disease [62]. Subsequent studies demonstrated the essential role of endogenous TLR7, but not TLR9, as a critical B cell-intrinsic sensor for the development of a spontaneous GC response in non-autoimmune and autoimmune environments [44, 45]. Altogether, these studies firmly established a direct causal link between intrinsic expression of *Tlr7* within B cells and the development of many autoimmune traits associated with SLE [44, 45, 62], underscoring the importance of tight regulation of TLR7 expression to prevent spontaneous autoimmunity.

## X chromosome dosage and SLE susceptibility

The X chromosome encompasses 155 Mb and over 1000 genes (47), including many protein-coding and miRNA genes known to shape the immune response [1, 9, 63]. Loss-of-function mutations in some of these genes (e.g., *BTK*, *WAS*, *IL2RG*, *FOXP3*) cause X-linked primary immunodeficiencies [1]. The genes of two RNA-sensing endosomal TLRs, *TLR7* and *TLR8*, are carried on a non-pseudoautosomal region of the short arm of the X chromosome (Xp) (Fig. 1).

X disomy or polysomy is thought to bring about a greater risk of developing autoimmunity [64–66]. For SLE, the risk is ninefold higher in women (46,XX) than that in men (46,XY) [67]. Relative to healthy men, the supernumerary X is associated with 15-fold greater susceptibility to SLE in men with Klinefelter syndrome (47,XXY) and 25-fold greater susceptibility in women with triple X syndrome (47,XXX) [68]. Furthermore, although many autoimmune diseases have been reported in women with Turner syndrome (45,X0), these patients rarely develop SLE [69].

The effect of the sex chromosome complement on autoimmune disease susceptibility has been addressed specifically using a set of mouse strains known as the four core genotypes [70]. In this model, the testes-determining *Sry* gene was deleted from the Y chromosome ($Y^-$), and ovary-bearing animals of either XX or $XY^-$ karyotype were generated. Backcrossing these females to males expressing a *Sry* transgene autosomally resulted in XX *Sry* and $XY^-$ *Sry* testes-bearing animals. A comparison was thus possible between XX and $XY^-$ mice against a shared female hormonal background and between XX *Sry* and $XY^-$ *Sry* animals of a male hormonal background [70, 71]. Relative to XY carriers, the XX sex chromosome complement was associated with enhanced susceptibility to pristane-induced [70] and spontaneous lupus [72].

Several genetic mechanisms could contribute independently to the effect of the sex chromosomes on autoimmune disease susceptibility: (1) differences in the dosage of pseudoautosomal genes; (2) differences in X-linked gene expression arising from the maternal or paternal inheritance of the chromosome, (3) genes carried by the Y chromosome, whose role in non-reproductive tissues is poorly known [73–76]; and (4) differences in X-linked gene expression due to escape from XCI.

## TLR7 escapes from X chromosome inactivation

Besides the four core genotypes mouse model, where the effect of X chromosome dosage is exerted over the entire organism [70, 72, 77], very few studies have examined the cell-intrinsic impact of the X chromosome complement on the functional response of well-characterized immune cell subsets

[78]. pDCs have recently emerged as a key DC subset specializing in type I IFN production. These cells play a critical role in SLE pathogenesis [47, 48], and their TLR7-driven functional response is strongly sex biased [79, 80]. Several studies have independently reported that pDCs from women produce more IFN-α than male pDCs upon stimulation with synthetic ligands or ssRNA that selectively activate TLR7 [79, 80]. This was shown to arise not from a sex bias in the level of IFN-α produced by individual cells, but from a higher frequency of IFN-α-secreting pDCs among female blood mononuclear cells [80, 81]. According to later reports, exogenous and endogenous estrogens increased TLR7-mediated IFN-α secretion by pDCs through cell-intrinsic estrogen receptor (ER) α signaling [81–83], but a role for the X chromosome complement could not be excluded. Our laboratory studied the respective contributions of X chromosome dosage and sex hormones in a humanized mouse model in which male and female NOD-SCID-β2m$^{-/-}$ animals received transplants of human CD34$^{+}$ progenitor cells from cord blood of either male or female donors. The human pDCs that developed in the bone marrow of the humanized male and female mice were subsequently assessed ex vivo for their capacity to produce cytokines in response to TLR7 and TLR8 ligands such as influenza virus and ssRNA ligands derived from the human immunodeficiency virus (HIV). As expected, the frequency of human pDCs producing IFN-α and TNF-α in response to TLR7 ligands was greater in female than that in male mice, suggesting a positive regulation by estrogens as previously reported [81–83]. Indeed, using an in vitro model of human pDC differentiation from CD34$^{+}$ progenitors, we found that blocking estrogen receptor signaling with the selective estrogen receptor degrader Fulvestrant ($ICI_{182,780}$) was able to blunt the TLR7-driven production of type I IFN and TNF-α by human pDCs [78]. Remarkably, when the TLR7-driven responses of pDCs were analyzed according to the sex of the human CD34$^{+}$ progenitor cells, pDCs from female donors were found to exhibit enhanced frequencies of IFN-α-producing cells compared to pDCs that developed from male CD34$^{+}$ progenitors. This enhanced responsiveness of female (XX) pDCs compared to male (XY) ones was not affected by the sex of the recipients, as similar differences were observed in both male and female humanized mice. These data provided the first evidence for a critical role of X chromosome dosage on the functional competence of woman pDCs, and suggested that the X chromosome complement and sex hormone estrogens, in concert but also independently, confer elevated production of type I IFN by women's pDCs in response to TLR7 ligands [78].

Recent work from our laboratory has investigated whether the human *TLR7* gene escapes XCI in primary immune cell populations from women, and the causal relationship between *TLR7* biallelic transcription and enhanced functional responses in individual cells [12]. Using a single-cell RT-PCR approach, we profiled the mono- or biallelic expression of *TLR7* by measuring the relative expression of *TLR7* transcripts from both X chromosomes in females heterozygous for *TLR7* exonic SNP markers. We showed that *TLR7* does escape X chromosome inactivation in distinct populations of immune cells, namely, pDCs, B cells, and monocytes from women. A proportion of cells exhibiting biallelic expression of *TLR7* was observed in all donors tested, with frequencies ranging from 7 to 45%. We also developed an RNA FISH approach to visualize in situ the biallelic expression of *TLR7* transcripts, using a XIST probe to identify the inactive X chromosome. We documented in this way the presence of primary *TLR7* transcripts on the inactive XIST-painted X chromosome of B lymphocytes, monocytes, and pDCs. Biallelic *TLR7* expression was associated with increased *TLR7* mRNA in naïve individual B lymphocytes, and probably accounted for the greater expression of TLR7 protein in the leukocytes from women than in the male cells. The elevated TLR7 protein dose in blood mononuclear cells from women correlated with an enhanced TLR7-driven proliferative response of PC lineage-committed B cells in women compared to men. By contrast, no sex bias was found when a ligand of TLR9 (which is autosomally encoded) was used to promote B cell proliferation. In women, B cells with biallelic *TLR7* expression were enriched within the CD27$^{hi}$ proliferating PC populations following treatment with TLR7-specific ligands. In support of causality, an increase in biallelic cell frequency was observed only in TLR7-stimulated cells, whereas counterselection of biallelic cells was instead observed in B cells that responded to a TLR9 ligand. This demonstrated that the increase in *TLR7*-biallelic B cells was not secondary to cell proliferation and supported the conclusion that biallelic B cells are endowed with enhanced responsiveness to a TLR7 ligand. Furthermore, using an in vitro model of naïve B cell differentiation [54], a causal link between biallelic expression of TLR7 and IgG class switch was also established [12]. Biallelic B cells again displayed over twofold greater propensity to class switch relative to monoallelic B cells in this assay, whereas no association was found when B cells were stimulated through TLR9 [12]. Altogether, this work demonstrated that the biallelic B cells of women are more responsive than monoallelic cells at specific checkpoints that involve signaling through TLR7 (Fig. 2). The cumulative effect of biallelic TLR7 dosage on B cell differentiation and PC proliferation during antigen-specific development of the B cell response could enhance the antibody response to ribonucleoprotein in women with SLE (Fig. 2), and also the normal antibody response to RNA viruses [84]. Whether this mechanism is indeed at play in female mammals warrants further investigations.

A strong indication that the number of X chromosomes, not just sex itself, is important in lupus comes from Klinefelter syndrome males, who carry one or more extra X

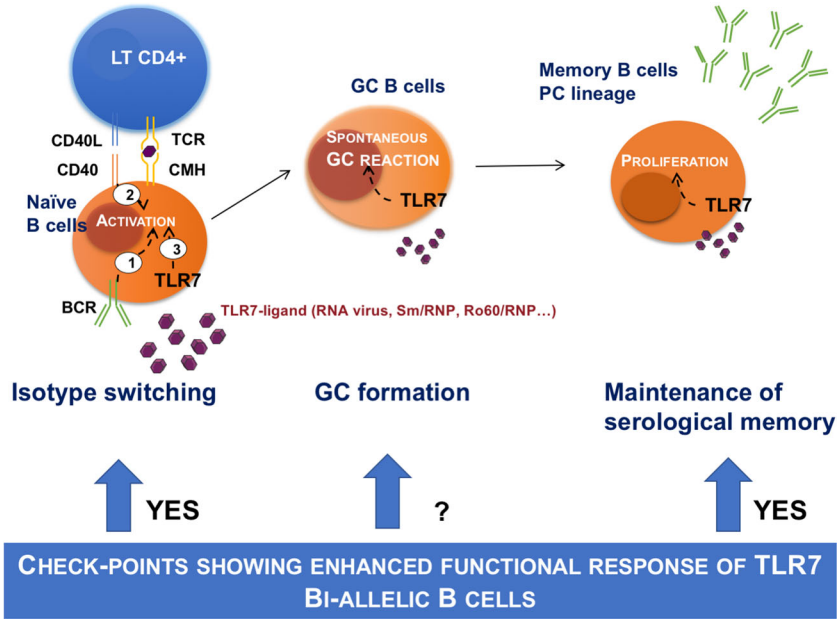


**Fig. 2** Biallelic B cells of women are more responsive than monoallelic cells at specific checkpoints that involve signaling through TLR7. TLR7 signaling has been reported to influence specific checkpoints of B cell activation and differentiation into GC B cells and memory B cells [36, 44, 45, 57–62]. We recently showed that the biallelic expression of *TLR7* is associated with a significant increase in *TLR7* mRNA in naïve single-cell B lymphocytes. Biallelic B lymphocytes were positively selected within $IgG^+$, class-switched B cells, in an in vitro activation and differentiation model, in response to TLR7—but not TLR9—specific ligands [12]. A significant enrichment in the frequency of *TLR7* biallelic $CD27^{hi}$ PC populations was found in B cells stimulated through TLR7 as compared to TLR9, strongly suggesting that *TLR7* gene dosage effect may preferentially drive the differentiation/expansion of committed PC-lineage B cells in women, relative to that in men [12]. The role of *TLR7* biallelic expression on the GC reaction is also plausible, given the central role of TLR7 in spontaneous GC formation [44, 45]; however, this will deserve further investigations

chromosomes and develop lupus and Sjögren's syndrome with a risk similar to that of females [64–66]. We also reported that *TLR7* escapes XCI in immune cells from 47,XXY Klinefelter syndrome males [12]. Because increased TLR7 is a known risk factor in lupus, we propose that higher TLR7 dosage arising from X-inactivation escape connects the presence of two X chromosomes in females and individuals with Klinefelter syndrome with the greater risk of developing lupus.

## Further genes evading X chromosome inactivation

Focusing on human lymphocyte populations, a recent study reported that some genes on the Xi are predisposed to become partially reactivated in the lymphocytes of women [11]. Biallelic expression of X-linked immune-related genes such as *CXCR3*, encoding a key chemokine receptor, and *CD40LG*, encoding the costimulatory molecule CD40 ligand, was documented in 4–5% of T cells and in EBV-transformed B cells. In agreement with this observation, another study found that *CD40LG*, which is repressed by methylation on the Xi of female cells, demethylates in the T cells of women with SLE; moreover, this was correlated with greater expression of *CD40LG* mRNA in T cells from female but not male SLE patients [13]. Both studies, however, documented the outcome of *CD40LG* XCI escape at the mRNA level, not at protein level, and the functional significance of this process is yet to be clarified.

TLR8 is encoded in the same region of the X chromosome as TLR7. The two genes are, in fact, adjacent to each other both in humans and in mice and are thought to originate from the duplication of an ancestral gene. In mice, five ectodomain amino acids are missing, which are known to be important for the recognition of ssRNA ligands by human TLR8 [85], and the functional character of the murine TLR8 protein is debated. Human TLR7 and TLR8 exhibit significant differences in their ligand recognition pattern. Human TLR8 preferentially recognizes ssRNA sequences rich in both A and U ribonucleotides, whereas sequences associating G and U ribonucleotides are preferential activators of TLR7 [86]. However, imidazoquinolines and nucleoside analogs can activate both TLRs, raising questions about the molecular basis for the recognition of such chemically and structurally distinct ligands [87]. The crystal structures of TLR7 and TLR8 [88, 89] have been solved recently, providing insights into the mechanisms of ligand recognition [87]. Both structures unexpectedly revealed two distinct binding sites on the receptor, specialized for different RNA degradation products [87]. The first site recognizes small agonists, uridine in TLR8 and guanosine in TLR7, and is essential for TLR activation. The second site of

TLR7 binds uridine-rich ssRNA, while the corresponding site on TLR8 binds U- and G-containing short ssRNA [87–89]. TLR7 thus appears as a dual sensor for guanosine and uridine-rich ssRNA, whereas TLR8 acts as a uridine sensor that is also able to sense ssRNA of diverse nucleoside composition [87].

Although the function of TLR8 in mice is not well understood [90], a recent study has examined the allelic expression of the *Tlr8* gene by RNA-FISH and suggests that it may escape XCI in female macrophages [14]. Whether *TLR8*, like *TLR7*, may escape XCI in human monocytes warrants further investigation. Human *TLR8* expression appears to be restricted to monocytes, myeloid dendritic cells, and neutrophils [91, 92]. *TLR8* can be also inadvertently expressed in other cell subsets in a disease context, e.g., in the pDCs of patients with systemic scleroderma, a life-threatening autoimmune disease that preferentially affects women [93]. Along the same line, human *TLR8* overexpression in mice has been shown to drive autoimmune inflammation and enhanced susceptibility to arthritis [92]. Interestingly, another recent study shows that sensing of viable pathogens by TLR8 in human monocytes provides key signals initiating follicular helper T cell differentiation [94]. Indeed, TLR8 agonists have been reported to possess unique adjuvant activity overcoming unresponsiveness to vaccines in newborns [95, 96], so that targeting TLR8 holds promise for improving the immunity elicited by vaccination. Whether *TLR8* evades XCI in immune cells, mirroring *TLR7*, and any functional consequences of *TLR8* biallelic expression in females, certainly deserve further investigation.

## Interactions between sex hormones and X-linked gene dosage

The female sex steroid hormones, estrogens, are a known factor of sexual dimorphism in immunity. For instance, B cell intrinsic ERα-signaling is able to control autoreactive B cell development and SLE in mice [97–100]. Moreover, as mentioned above, both estrogens and the number of X chromosomes in the karyotype independently contribute to the enhanced TLR7-mediated responses of pDCs from women [78]. The abundance of mRNA and protein gene products as a function of *TLR7* biallelic expression in individual female pDCs is currently unknown. As pDCs constitutively express noticeably higher levels of TLR7 protein than B cells or monocytes, one can speculate that *TLR7* biallelism in pDCs may have a limited functional impact due to the presence of optimal amounts of TLR7 protein in these cells. In this context, the higher responsiveness of female pDCs could depend on signaling intermediaries of the Myd88-signaling machinery or on helper molecules in TLR trafficking or maturation. In this respect, it has been reported recently that estrogen signaling upregulates the expression of the endoplasmic reticulum transmembrane protein Unc93b1 in immune cells [101]. Unc93b1 is a chaperone molecule regulating the subcellular localization of endosomal TLR3, TLR7, and TLR9, and Unc93b1 knockdown abrogates cytokine production [102]. It is yet unknown whether estrogen-mediated regulation of Unc93b1 occurs in pDCs specifically [101]. By contrast, a positive correlation between estradiol signaling through ERα and IRF5 expression in human and mouse pDCs has been reported [83]. Although the exact contribution of IRF5 to the production of type I IFN by TLR-stimulated pDCs is still debated, one can speculate that IRF5 upregulation by estrogens may enhance IFN-α production in synergy with IRF7 [103, 104].

Such modulatory effects of estrogen signaling might be necessary to unmask gene dosage effect due to XCI escape of genes like *TLR7*/*Tlr7*. This could be particularly relevant in B lymphocytes, where a large body of literature suggests that estrogens, via ERα-signaling, exert a powerful effect on B cells. Estrogens have indeed been reported to promote the expression of CD22 and SHP-1 in B cells, thereby decreasing the threshold for B cell receptor signaling and allowing the survival of autoreactive B cells through upregulation of BCL-2 and the survival factor BAFF [97] [98]. Moreover, estrogens have been suggested to promote somatic hypermutation by stimulating the expression of activation-induced cytidine deaminase [99]. Although these effects of estrogens were not shown to occur in vivo through cell-intrinsic ERα activation, an elegant study by Gould and colleagues recently reported that B cell-intrinsic ERα signaling partially controlled B cell activation and disease severity in (NZB × NZW) F1 mice [100]. The interaction of hormonal factors with the X chromosome complement, involving the effect of XCI escape, is probably a substantial contributor to the enhanced susceptibility of females to TLR7-dependent autoimmune diseases.

## Concluding remarks

The cause of SLE is not well understood but is thought to involve a complex interaction of genetic, environmental, and hormonal factors [105]. Although female sex hormones have been shown to regulate key immune cell subsets involved in SLE, such as B cells [97–100] and pDCs [78–83], the observation that Klinefelter syndrome men are as susceptible to SLE as women strongly suggested that the higher female sex hormone profile is not the sole contributor to the sex bias in SLE [64–66]. Since the seminal observations of *Tlr7* gene dosage as a key factor of lupus-like syndrome in mice [20–22], it was hypothesized that functional *TLR7* gene duplication in women due to XCI escape was a contributor to sex bias in SLE. The evidence we have reviewed supports the notion that the X chromosome itself constitutes an etiological factor. There is no longer any doubt that *TLR7* escapes from X chromosome inactivation in women and Klinefelter syndrome

men and that biallelic expression of *TLR7* has functional consequences on B cell responsiveness [12]. A wider examination of XCI escape is now necessary to encompass further X-linked genes encoding immune regulators. *FOXP3* has been reported not to evade XCI [106, 107], but there are indications that *CD40LG* may do so, though the functional consequences have not yet been addressed [11, 13]. Overall, we propose a major role for the XCI escape of *TLR7* in generating genomically determined, stochastic diversity within the female immune cell compartment, with a likely impact on innate and adaptive immunity against selected pathogens or self-components. We believe that the quantitative edge in *TLR7* expression generated by biallelism may affect immunity both qualitatively and quantitatively, augmenting the susceptibility of women and Klinefelter men to autoimmune diseases such as SLE and Sjögren's syndrome [64–66].

## Future directions

It is now critical to extend our investigations of XCI to clinical cohorts in SLE and other autoimmune disorders, in a comparison with healthy individuals. This represents a considerable technological challenge in that, so far, no specific markers exist to distinguish biallelic from monoallelic gene expression in single cells, apart from the analysis of *TLR7* transcripts using either allelic markers, as we reported recently [12], or RNA FISH. Both approaches are beset by specific limitations due to the low frequency of exonic polymorphisms of the *TLR7* locus; in addition, the conceivable impact of the few frequent polymorphisms on TLR7 functions, and hence on disease susceptibility, may be a confounding factor [108, 109]. Alternative unbiased approaches using RNA FISH are possible but may be painstaking to optimize for high throughput and high sensitivity. One recent study which examined the biallelic expression of *TLR7* in EBV-transformed B cell lines suggested that *TLR7* evades XCI also in women with SLE [11]. Although XCI escape has been reported in mice, previous surveys of XCI in various mouse cell lines showed that only 3% of mouse genes partially or completely escape inactivation [110–112]. None of these studies identified XCI escape of X-linked autoimmunity-related genes, probably because cell lines (e.g., fibroblasts) were used that do not express many of these tissue-specific genes. Whether immune-related X-linked genes escape XCI in mouse immune cells remains an open question deserving further investigation. Finally, our finding that *TLR7* escapes from XCI in immune cells sheds light on the sexual dimorphism of humoral immunity, particularly in the context of RNA virus infection [84], and may have broader implications for our understanding of the pathogenesis of RNA virus-mediated infectious diseases, such as HIV-1 infection and chronic hepatitis C, for which sex-based differences have been reported [2, 113].

**Author contributions** All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

**Funding** This work was supported by grants from Fondation pour la Recherche Médicale (DEQ20131029169 and DEQ20180339187), Fonds de Dotation CSL Behring, Fondation Arthritis, SIDACTION and Fondation ARC.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Libert C, Dejager L, Pinheiro I (2010) The X chromosome in immune functions: when a chromosome makes the difference. Nat Rev Immunol 10(8):594–604
2. Markle JG, Fish EN (2014) SeXX matters in immunity. Trends Immunol 35(3):97–104
3. Klein SL, Flanagan KL (2016) Sex differences in immune responses. Nat Rev Immunol 16(10):626–638
4. Laffont S, Garnier L, Lelu K, Guery JC (2015) Estrogen-mediated protection of experimental autoimmune encephalomyelitis: lessons from the dissection of estrogen receptor-signaling in vivo. Biom J 38(3):194–205
5. Laffont S, Seillet C, Guery JC (2017) Estrogen receptor-dependent regulation of dendritic cell development and function. Front Immunol 8:108
6. Laffont S, Blanquart E, Guery JC (2017) Sex differences in asthma: a key role of androgen-signaling in group 2 innate lymphoid cells. Front Immunol 8:1069
7. Augui S, Nora EP, Heard E (2011) Regulation of X-chromosome inactivation by the X-inactivation centre. Nat Rev Genet 12(6):429–442
8. Chaumeil J, Augui S, Chow JC, Heard E (2008) Combined immunofluorescence, RNA fluorescent in situ hybridization, and DNA fluorescent in situ hybridization to study chromatin changes, transcriptional activity, nuclear organization, and X-chromosome inactivation. Methods Mol Biol 463:297–308
9. Fish EN (2008) The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8(9):737–744
10. Carrel L, Willard HF (2005) X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434(7031):400–404
11. Wang J, Syrett CM, Kramer MC, Basu A, Atchison ML, Anguera MC (2016) Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X. Proc Natl Acad Sci U S A 113(14):E2029–E2038
12. Souyris M, Cenac C, Azar P, Daviaud D, Canivet A, Grunenwald S, Pienkowski C, Chaumeil J, Mejia JE, Guery JC (2018) TLR7 escapes X chromosome inactivation in immune cells. Sci Immunol 3(19):eaap8855
13. Lu Q, Wu A, Tesmer L, Ray D, Yousif N, Richardson B (2007) Demethylation of CD40LG on the inactive X in T cells from women with lupus. J Immunol 179(9):6352–6358
14. McDonald G, Cabal N, Vannier A, Umiker B, Yin RH, Orjalo AV Jr, Johansson HE, Han JH, Imanishi-Kari T (2015) Female bias in systemic lupus erythematosus is associated with the differential expression of X-linked Toll-like receptor 8. Front Immunol 6:457
15. Savarese F, Flahndorfer K, Jaenisch R, Busslinger M, Wutz A (2006) Hematopoietic precursor cells transiently reestablish permissiveness for X inactivation. Mol Cell Biol 26(19):7167–7177

16. Syrett CM, Sindhava V, Hodawadekar S, Myles A, Liang G, Zhang Y, Nandi S, Cancro M, Atchison M, Anguera MC (2017) Loss of Xist RNA from the inactive X during B cell development is restored in a dynamic YY1-dependent two-step process in activated B cells. PLoS Genet 13(10):e1007050
17. Qu K, Zaba LC, Giresi PG, Li R, Longmire M, Kim YH, Greenleaf WJ, Chang HY (2015) Individuality and variation of personal regulomes in primary human T cells. Cell Syst 1(1):51–61
18. Theofilopoulos AN, Kono DH, Baccala R (2017) The multiple pathways to autoimmunity. Nat Immunol 18(7):716–724
19. Green NM, Marshak-Rothstein A (2011) Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. Semin Immunol 23(2):106–112
20. Subramanian S, Tus K, Li QZ, Wang A, Tian XH, Zhou J, Liang C, Bartov G, McDaniel LD, Zhou XJ, Schultz RA, Wakeland EK (2006) A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. Proc Natl Acad Sci U S A 103(26):9970–9975
21. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S (2006) Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 312(5780):1669–1672
22. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM, Flavell RA, Bolland S (2007) Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity 27(5):801–810
23. Petri M, Orbai AM, Alarcón Graciela S, Gordon C, Merrill Joan T, Fortin Paul R, Bruce Ian N, Isenberg D, Wallace Daniel J, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly John G, Sánchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D, Kalunian K, Costner M, Werth Victoria P, Zoma A, Bernatsky S, Ruiz-Irastorza G, Khamashta Munther A, Jacobsen S, Buyon Jill P, Maddison P, Dooley Mary A, van Vollenhoven Ronald F, Ginzler E, Stoll T, Peschken C, Jorizzo Joseph L, Callen Jeffrey P, Lim SS, Fessler Barri J, Inanc M, Kamen Diane L, Rahman A, Steinsson K, Franks Andrew G, Sigler L, Hameed S, Fang H, Pham N, Brey R, Weisman Michael H, McGwin G, Magder Laurence S (2012) Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum 64(8):2677–2686
24. Hua J, Kirou K, Lee C, Crow MK (2006) Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. Arthritis Rheum 54(6):1906–1916
25. Hooks JJ, Moutsopoulos HM, Geis SA, Stahl NI, Decker JL, Notkins AL (1979) Immune interferon in the circulation of patients with autoimmune disease. N Engl J Med 301(1):5–8
26. Ytterberg SR, Schnitzer TJ (1982) Serum interferon levels in patients with systemic lupus erythematosus. Arthritis Rheum 25(4):401–406
27. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 100(5):2610–2615
28. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, Pascual V (2003) Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 197(6):711–723
29. Kirou KA, Lee C, George S, Louca K, Papagiannis IG, Peterson MG, Ly N, Woodward RN, Fry KE, Lau AY, Prentice JG, Wohlgemuth JG, Crow MK (2004) Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus. Arthritis Rheum 50(12):3958–3967
30. Gilliet M, Cao W, Liu YJ (2008) Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol 8(8):594–606
31. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J (2001) Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science 294(5546):1540–1543
32. Marshak-Rothstein A (2006) Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol 6(11):823–835
33. Swiecki M, Colonna M (2015) The multifaceted biology of plasmacytoid dendritic cells. Nat Rev Immunol 15(8):471–485
34. Banchereau J, Pascual V (2006) Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity 25(3):383–392
35. Simchoni N, Cunningham-Rundles C (2015) TLR7- and TLR9-responsive human B cells share phenotypic and genetic characteristics. J Immunol 194(7):3035–3044
36. Hung T, Pratt GA, Sundararaman B, Townsend MJ, Chaivorapol C, Bhangale T, Graham RR, Ortmann W, Criswell LA, Yeo GW, Behrens TW (2015) The Ro60 autoantigen binds endogenous retroelements and regulates inflammatory gene expression. Science 350(6259):455–459
37. Green NM, Laws A, Kiefer K, Busconi L, Kim YM, Brinkmann MM, Trail EH, Yasuda K, Christensen SR, Shlomchik MJ, Vogel S, Connor JH, Ploegh H, Eilat D, Rifkin IR, van Seventer JM, Marshak-Rothstein A (2009) Murine B cell response to TLR7 ligands depends on an IFN-beta feedback loop. J Immunol 183(3):1569–1576
38. Rawlings DJ, Schwartz MA, Jackson SW, Meyer-Bahlburg A (2012) Integration of B cell responses through Toll-like receptors and antigen receptors. Nat Rev Immunol 12(4):282–294
39. Fairhurst AM, Hwang SH, Wang A, Tian XH, Boudreaux C, Zhou XJ, Casco J, Li QZ, Connolly JE, Wakeland EK (2008) Yaa autoimmune phenotypes are conferred by overexpression of TLR7. Eur J Immunol 38(7):1971–1978
40. Santiago-Raber ML, Kikuchi S, Borel P, Uematsu S, Akira S, Kotzin BL, Izui S (2008) Evidence for genes in addition to Tlr7 in the Yaa translocation linked with acceleration of systemic lupus erythematosus. J Immunol 181(2):1556–1562
41. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ (2006) Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity 25(3):417–428
42. Walsh ER, Pisitkun P, Voynova E, Deane JA, Scott BL, Caspi RR, Bolland S (2012) Dual signaling by innate and adaptive immune receptors is required for TLR7-induced B-cell-mediated autoimmunity. Proc Natl Acad Sci U S A 109(40):16276–16281
43. Anderson KG, Mayer-Barber K, Sung H, Beura L, James BR, Taylor JJ, Qunaj L, Griffith TS, Vezys V, Barber DL, Masopust D (2014) Intravascular staining for discrimination of vascular and tissue leukocytes. Nat Protoc 9(1):209–222
44. Jackson SW, Scharping NE, Kolhatkar NS, Khim S, Schwartz MA, Li QZ, Hudkins KL, Alpers CE, Liggitt D, Rawlings DJ (2014) Opposing impact of B cell-intrinsic TLR7 and TLR9 signals on autoantibody repertoire and systemic inflammation. J Immunol 192(10):4525–4532
45. Soni C, Wong EB, Domeier PP, Khan TN, Satoh T, Akira S, Rahman ZS (2014) B cell-intrinsic TLR7 signaling is essential for the development of spontaneous germinal centers. J Immunol 193(9):4400–4414
46. Ronnblom L (2011) The type I interferon system in the etiopathogenesis of autoimmune diseases. Ups J Med Sci 116(4):227–237
47. Rowland SL, Riggs JM, Gilfillan S, Bugatti M, Vermi W, Kolbeck R, Unanue ER, Sanjuan MA, Colonna M (2014) Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. J Exp Med 211(10):1977–1991
48. Sisirak V, Ganguly D, Lewis KL, Couillault C, Tanaka L, Bolland S, D'Agati V, Elkon KB, Reizis B (2014) Genetic evidence for the role of plasmacytoid dendritic cells in systemic lupus erythematosus. J Exp Med 211(10):1969–1976

49. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchereau J, Pascual V (2011) Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 3(73):73ra20
50. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, Meller S, Chamilos G, Sebasigari R, Riccieri V, Bassett R, Amuro H, Fukuhara S, Ito T, Liu YJ, Gilliet M (2011) Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med 3(73):73ra19
51. Craft JE (2011) Dissecting the immune cell mayhem that drives lupus pathogenesis. Sci Transl Med 3(73):73ps9
52. Jacquemin C, Schmitt N, Contin-Bordes C, Liu Y, Narayanan P, Seneschal J, Maurouard T, Dougall D, Davizon ES, Dumortier H, Douchet I, Raffray L, Richez C, Lazaro E, Duffau P, Truchetet ME, Khoryati L, Mercie P, Couzi L, Merville P, Schaeverbeke T, Viallard JF, Pellegrin JL, Moreau JF, Muller S, Zurawski S, Coffman RL, Pascual V, Ueno H, Blanco P (2015) OX40 ligand contributes to human lupus pathogenesis by promoting T follicular helper response. Immunity 42(6):1159–1170
53. Pasare C, Medzhitov R (2005) Control of B-cell responses by Toll-like receptors. Nature 438(7066):364–368
54. Ruprecht CR, Lanzavecchia A (2006) Toll-like receptor stimulation as a third signal required for activation of human naive B cells. Eur J Immunol 36(4):810–816
55. Hwang IY, Park C, Harrison K, Kehrl JH (2009) TLR4 signaling augments B lymphocyte migration and overcomes the restriction that limits access to germinal center dark zones. J Exp Med 206(12):2641–2657
56. Bernasconi NL, Traggiai E, Lanzavecchia A (2002) Maintenance of serological memory by polyclonal activation of human memory B cells. Science 298(5601):2199–2202
57. Browne EP (2011) Toll-like receptor 7 controls the anti-retroviral germinal center response. PLoS Pathog 7(10):e1002293
58. DeFranco AL, Rookhuizen DC, Hou B (2012) Contribution of Toll-like receptor signaling to germinal center antibody responses. Immunol Rev 247(1):64–72
59. Mankan AK, Hornung V (2012) Retroviral danger from within: TLR7 is in control. Immunity 37(5):763–766
60. Hou B, Saudan P, Ott G, Wheeler ML, Ji M, Kuzmich L, Lee LM, Coffman RL, Bachmann MF, DeFranco AL (2011) Selective utilization of Toll-like receptor and MyD88 signaling in B cells for enhancement of the antiviral germinal center response. Immunity 34(3):375–384
61. Lau CM, Broughton C, Tabor AS, Akira S, Flavell RA, Mamula MJ, Christensen SR, Shlomchik MJ, Viglianti GA, Rifkin IR, Marshak-Rothstein A (2005) RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. J Exp Med 202(9):1171–1177
62. Hwang SH, Lee H, Yamamoto M, Jones LA, Dayalan J, Hopkins R, Zhou XJ, Yarovinsky F, Connolly JE, Curotto de Lafaille MA, Wakeland EK, Fairhurst AM (2012) B cell TLR7 expression drives anti-RNA autoantibody production and exacerbates disease in systemic lupus erythematosus-prone mice. J Immunol 189(12): 5786–5796
63. Pinheiro I, Dejager L, Libert C (2011) X-chromosome-located microRNAs in immunity: might they explain male/female differences? The X chromosome-genomic context may affect X-located miRNAs and downstream signaling, thereby contributing to the enhanced immune response of females. Bioessays 33(11):791–802
64. Scofield RH, Bruner GR, Namjou B, Kimberly RP, Ramsey-Goldman R, Petri M, Reveille JD, Alarcon GS, Vila LM, Reid J, Harris B, Li S, Kelly JA, Harley JB (2008) Klinefelter's syndrome (47,XXY) in male systemic lupus erythematosus patients: support for the notion of a gene-dose effect from the X chromosome. Arthritis Rheum 58(8):2511–2517
65. Harris VM, Sharma R, Cavett J, Kurien BT, Liu K, Koelsch KA, Rasmussen A, Radfar L, Lewis D, Stone DU, Kaufman CE, Li S, Segal B, Wallace DJ, Weisman MH, Venuturupalli S, Kelly JA, Alarcon-Riquelme ME, Pons-Estel B, Jonsson R, Lu X, Gottenberg JE, Anaya JM, Cunninghame-Graham DS, Huang AJW, Brennan MT, Hughes P, Alevizos I, Miceli-Richard C, Keystone EC, Bykerk VP, Hirschfield G, Xie G, Ng WF, Nordmark G, Bucher SM, Eriksson P, Omdal R, Rhodus NL, Rischmueller M, Rohrer M, Wahren-Herlenius M, Witte T, Mariette X, Lessard CJ, Harley JB, Sivils KL, Scofield RH (2016) Klinefelter's syndrome (47,XXY) is in excess among men with Sjögren's syndrome. Clin. Immunol 168:25–29
66. Seminog OO, Seminog AB, Yeates D, Goldacre MJ (2015) Associations between Klinefelter's syndrome and autoimmune diseases: English national record linkage studies. Autoimmunity 48(2):125–128
67. Whitacre CC (2001) Sex differences in autoimmune disease. Nat Immunol 2(9):777–780
68. Liu K, Kurien BT, Zimmerman SL, Kaufman KM, Taft DH, Kottyan LC, Lazaro S, Weaver CA, Ice JA, Adler AJ, Chodosh J, Radfar L, Rasmussen A, Stone DU, Lewis DM, Li S, Koelsch KA, Igoe A, Talsania M, Kumar J, Maier-Moore JS, Harris VM, Gopalakrishnan R, Jonsson R, Lessard JA, Lu X, Gottenberg JE, Anaya JM, Cunninghame-Graham DS, Huang AJW, Brennan MT, Hughes P, Illei GG, Miceli-Richard C, Keystone EC, Bykerk VP, Hirschfield G, Xie G, Ng WF, Nordmark G, Eriksson P, Omdal R, Rhodus NL, Rischmueller M, Rohrer M, Segal BM, Vyse TJ, Wahren-Herlenius M, Witte T, Pons-Estel B, Alarcon-Riquelme ME, Guthridge JM, James JA, Lessard CJ, Kelly JA, Thompson SD, Gaffney PM, Montgomery CG, Edberg JC, Kimberly RP, Alarcon GS, Langefeld CL, Gilkeson GS, Kamen DL, Tsao BP, McCune WJ, Salmon JE, Merrill JT, Weisman MH, Wallace DJ, Utset TO, Bottinger EP, Amos CI, Siminovitch KA, Mariette X, Sivils KL, Harley JB, Scofield RH (2016) X chromosome dose and sex bias in autoimmune diseases: increased prevalence of 47,XXX in systemic lupus erythematosus and Sjogren's syndrome. Arthritis Rheumatol 68(5):1290–1300
69. Bai J, Qiao J, Wu Y, Zhao Z, Fang H (2015) Systemic lupus erythematosus in a patient with Turner syndrome. An Bras Dermatol 90(4):600–601
70. Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, Arnold AP, Singh RR, Voskuhl RR (2008) A role for sex chromosome complement in the female bias in autoimmune disease. J Exp Med 205(5):1099–1108
71. Arnold AP, Burgoyne PS (2004) Are XX and XY brain cells intrinsically different? Trends Endocrinol Metab 15(1):6–11
72. Sasidhar MV, Itoh N, Gold SM, Lawson GW, Voskuhl RR (2012) The XX sex chromosome complement in mice is associated with increased spontaneous lupus compared with XY. Ann Rheum Dis 71(8):1418–1422
73. Teuscher C, Noubade R, Spach K, McElvany B, Bunn JY, Fillmore PD, Zachary JF, Blankenhorn EP (2006) Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A 103(21):8024–8029
74. Spach KM, Blake M, Bunn JY, McElvany B, Noubade R, Blankenhorn EP, Teuscher C (2009) Cutting edge: the Y chromosome controls the age-dependent experimental allergic encephalomyelitis sexual dimorphism in SJL/J mice. J Immunol 182(4): 1789–1793
75. Case LK, Wall EH, Dragon JA, Saligrama N, Krementsov DN, Moussawi M, Zachary JF, Huber SA, Blankenhorn EP, Teuscher C (2013) The Y chromosome as a regulatory element shaping immune cell transcriptomes and susceptibility to autoimmune disease. Genome Res 23(9):1474–1485

76. Charchar FJ, Bloomer LDS, Barnes TA, Cowley MJ, Nelson CP, Wang Y, Denniff M, Debiec R, Christofidou P, Nankervis S, Dominiczak AF, Bani-Mustafa A, Balmforth AJ, Hall AS, Erdmann J, Cambien F, Deloukas P, Hengstenberg C, Packard C, Schunkert H, Ouwehand WH, Ford I, Goodall AH, Jobling MA, Samani NJ, Tomaszewski M (2012) Inheritance of coronary artery disease in men: an analysis of the role of the Y chromosome. Lancet 379(9819):915–922
77. Du S, Itoh N, Askarinam S, Hill H, Arnold AP, Voskuhl RR (2014) XY sex chromosome complement, compared with XX, in the CNS confers greater neurodegeneration during experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 111(7):2806–2811
78. Laffont S, Rouquie N, Azar P, Seillet C, Plumas J, Aspord C, Guery JC (2014) X-Chromosome complement and estrogen receptor signaling independently contribute to the enhanced TLR7-mediated IFN-alpha production of plasmacytoid dendritic cells from women. J Immunol 193(11):5444–5452
79. Berghofer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H (2006) TLR7 ligands induce higher IFN-alpha production in females. J Immunol 177(4):2088–2096
80. Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, Wen TF, Lindsay RJ, Orellana L, Mildvan D, Bazner S, Streeck H, Alter G, Lifson JD, Carrington M, Bosch RJ, Robbins GK, Altfeld M (2009) Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med 15(8): 955–959
81. Seillet C, Laffont S, Tremollieres F, Rouquie N, Ribot C, Arnal JF, Douin-Echinard V, Gourdy P, Guery JC (2012) The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor alpha signaling. Blood 119(2):454–464
82. Seillet C, Rouquie N, Foulon E, Douin-Echinard V, Krust A, Chambon P, Arnal JF, Guery JC, Laffont S (2013) Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor alpha. J Immunol 190(11):5459–5470
83. Griesbeck M, Ziegler S, Laffont S, Smith N, Chauveau L, Tomezsko P, Sharei A, Kourjian G, Porichis F, Hart M, Palmer CD, Sirignano M, Beisel C, Hildebrandt H, Cenac C, Villani AC, Diefenbach TJ, Le Gall S, Schwartz O, Herbeuval JP, Autran B, Guery JC, Chang JJ, Altfeld M (2015) Sex differences in plasmacytoid dendritic cell levels of IRF5 drive higher IFN-alpha production in women. J Immunol 195(11):5327–5336
84. Fink AL, Klein SL (2018) The evolution of greater humoral immunity in females than males: implications for vaccine efficacy. Current Opinion in Physiology 6:16–20
85. Liu J, Xu C, Hsu LC, Luo Y, Xiang R, Chuang TH (2010) A five-amino-acid motif in the undefined region of the TLR8 ectodomain is required for species-specific ligand recognition. Mol Immunol 47(5):1083–1090
86. Forsbach A, Nemorin JG, Montino C, Muller C, Samulowitz U, Vicari AP, Jurk M, Mutwiri GK, Krieg AM, Lipford GB, Vollmer J (2008) Identification of RNA sequence motifs stimulating sequence-specific TLR8-dependent immune responses. J Immunol 180(6):3729–3738
87. Shimizu T (2017) Structural insights into ligand recognition and regulation of nucleic acid-sensing Toll-like receptors. Curr Opin Struct Biol 47:52–59
88. Tanji H, Ohto U, Shibata T, Taoka M, Yamauchi Y, Isobe T, Miyake K, Shimizu T (2015) Toll-like receptor 8 senses degradation products of single-stranded RNA. Nat Struct Mol Biol 22(2): 109–115
89. Zhang Z, Ohto U, Shibata T, Krayukhina E, Taoka M, Yamauchi Y, Tanji H, Isobe T, Uchiyama S, Miyake K, Shimizu T (2016) Structural analysis reveals that Toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. Immunity 45(4):737–748
90. Desnues B, Macedo AB, Roussel-Queval A, Bonnardel J, Henri S, Demaria O, Alexopoulou L (2014) TLR8 on dendritic cells and TLR9 on B cells restrain TLR7-mediated spontaneous autoimmunity in C57BL/6 mice. Proc Natl Acad Sci U S A 111(4):1497–1502
91. Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G (2002) Quantitative expression of Toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168(9):4531–4537
92. Guiducci C, Gong M, Cepika AM, Xu Z, Tripodo C, Bennett L, Crain C, Quartier P, Cush JJ, Pascual V, Coffman RL, Barrat FJ (2013) RNA recognition by human TLR8 can lead to autoimmune inflammation. J Exp Med 210(13):2903–2919
93. Kioon MDA, Tripodo C, Fernandez D, Kirou KA, Spiera RF, Crow MK, Gordon JK, Barrat FJ (2018) Plasmacytoid dendritic cells promote systemic sclerosis with a key role for TLR8. Sci Transl Med 10:423
94. Ugolini M, Gerhard J, Burkert S, Jensen KJ, Georg P, Ebner F, Volkers SM, Thada S, Dietert K, Bauer L, Schafer A, Helbig ET, Opitz B, Kurth F, Sur S, Dittrich N, Gaddam S, Conrad ML, Benn CS, Blohm U, Gruber AD, Hutloff A, Hartmann S, Boekschoten MV, Muller M, Jungersen G, Schumann RR, Suttorp N, Sander LE (2018) Recognition of microbial viability via TLR8 drives TFH cell differentiation and vaccine responses. Nat Immunol 19(4):386–396
95. Dowling DJ, van Haren SD, Scheid A, Bergelson I, Kim D, Mancuso CJ, Foppen W, Ozonoff A, Fresh L, Theriot TB, Lackner AA, Fichorova RN, Smirnov D, Vasilakos JP, Beaurline JM, Tomai MA, Midkiff CC, Alvarez X, Blanchard JL, Gilbert MH, Aye PP, Levy O (2017) TLR7/8 adjuvant overcomes newborn hyporesponsiveness to pneumococcal conjugate vaccine at birth. JCI Insight 2(6):e91020
96. Dowling DJ, Scott EA, Scheid A, Bergelson I, Joshi S, Pietrasanta C, Brightman S, Sanchez-Schmitz G, Van Haren SD, Ninkovic J, Kats D, Guiducci C, de Titta A, Bonner DK, Hirosue S, Swartz MA, Hubbell JA, Levy O (2017) Toll-like receptor 8 agonist nanoparticles mimic immunomodulating effects of the live BCG vaccine and enhance neonatal innate and adaptive immune responses. J Allergy Clin Immunol 140(5):1339–1350
97. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B (2002) Estrogen alters thresholds for B cell apoptosis and activation. J Clin Invest 109(12):1625–1633
98. Hill L, Jeganathan V, Chinnasamy P, Grimaldi C, Diamond B (2011) Differential roles of estrogen receptors alpha and beta in control of B-cell maturation and selection. Mol Med 17(3–4):211–220
99. Pauklin S, Sernandez IV, Bachmann G, Ramiro AR, Petersen-Mahrt SK (2009) Estrogen directly activates AID transcription and function. J Exp Med 206(1):99–111
100. Tabor DE, Gould KA (2017) Estrogen receptor alpha promotes lupus in (NZBxNZW)F1 mice in a B cell intrinsic manner. Clin Immunol 174:41–52
101. Panchanathan R, Liu H, Choubey D (2013) Expression of murine Unc93b1 is up-regulated by interferon and estrogen signaling: implications for sex bias in the development of autoimmunity. Int Immunol 25(9):521–529
102. Tabeta K, Hoebe K, Janssen EM, Du X, Georgel P, Crozat K, Mudd S, Mann N, Sovath S, Goode J, Shamel L, Herskovits AA, Portnoy DA, Cooke M, Tarantino LM, Wiltshire T, Steinberg BE, Grinstein S, Beutler B (2006) The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. Nat Immunol 7(2):156–164

103. Pelka K, Latz E (2013) IRF5, IRF8, and IRF7 in human pDCs—the good, the bad, and the insignificant? Eur J Immunol 43(7): 1693–1697
104. Steinhagen F, McFarland AP, Rodriguez LG, Tewary P, Jarret A, Savan R, Klinman DM (2013) IRF-5 and NF-kappaB p50 co-regulate IFN-beta and IL-6 expression in TLR9-stimulated human plasmacytoid dendritic cells. Eur J Immunol 43(7):1896–1906
105. Tsokos GC, Lo MS, Reis PC, Sullivan KE (2016) New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol 12:716–730
106. Cotton AM, Lam L, Affleck JG, Wilson IM, Peñaherrera MS, McFadden DE, Kobor MS, Lam WL, Robinson WP, Brown CJ (2011) Chromosome-wide DNA methylation analysis predicts human tissue-specific X inactivation. Hum Genet 130(2):187–201
107. Di Nunzio S, Cecconi M, Passerini L, McMurchy AN, Baron U, Turbachova I, Vignola S, Valencic E, Tommasini A, Junker A, Cazzola G, Olek S, Levings MK, Perroni L, Roncarolo MG, Bacchetta R (2009) Wild-type FOXP3 is selectively active in CD4+CD25(hi) regulatory T cells of healthy female carriers of different FOXP3 mutations. Blood 114(19):4138–4141
108. Deng Y, Zhao J, Sakurai D, Kaufman KM, Edberg JC, Kimberly RP, Kamen DL, Gilkeson GS, Jacob CO, Scofield RH, Langefeld CD, Kelly JA, Ramsey-Goldman R, Petri MA, Reveille JD, Vila LM, Alarcon GS, Vyse TJ, Pons-Estel BA, Freedman BI, Gaffney PM, Sivils KM, James JA, Gregersen PK, Anaya JM, Niewold TB, Merrill JT, Criswell LA, Stevens AM, Boackle SA, Cantor RM, Chen W, Grossman JM, Hahn BH, Harley JB, Alarcomicronn-Riquelme ME, Brown EE, Tsao BP (2013) MicroRNA-3148 modulates allelic expression of toll-like receptor 7 variant associated with systemic lupus erythematosus. PLoS Genet 9(2):e1003336
109. Oh DY, Baumann K, Hamouda O, Eckert JK, Neumann K, Kucherer C, Bartmeyer B, Poggensee G, Oh N, Pruss A, Jessen H, Schumann RR (2009) A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. Aids 23(3):297–307
110. Yang F, Babak T, Shendure J, Disteche CM (2010) Global survey of escape from X inactivation by RNA-sequencing in mouse. Genome Res 20(5):614–622
111. Pinter SF, Sadreyev RI, Yildirim E, Jeon Y, Ohsumi TK, Borowsky M, Lee JT (2012) Spreading of X chromosome inactivation via a hierarchy of defined Polycomb stations. Genome Res 22(10):1864–1876
112. Deng X, Ma W, Ramani V, Hill A, Yang F, Ay F, Berletch JB, Blau CA, Shendure J, Duan Z, Noble WS, Disteche CM (2015) Bipartite structure of the inactive mouse X chromosome. Genome Biol 16:152
113. van den Berg CH, Grady BP, Schinkel J, van de Laar T, Molenkamp R, van Houdt R, Coutinho RA, van Baarle D, Prins M (2011) Female sex and IL28B, a synergism for spontaneous viral clearance in hepatitis C virus (HCV) seroconverters from a community-based cohort. PLoS One 6(11):e27555