Intrinsic autoimmune capacities of hematopoietic cells from female New Zealand hybrid mice

A David1, A Trigunaite2, MK MacLeod1,2, AC Johnson2,4, P Marrack1 and TN Jørgensen1,2

Most systemic autoimmune diseases occur more frequently in females than in males. This is particularly evident in Sjögren’s syndrome, systemic lupus erythematosus (SLE) and thyroid autoimmunity, where the ratio of females to males ranges from 20:1 to 8:1. Our understanding of the etiology of SLE implies important roles for genetics, environmental factors and sex hormones, but the relative significance of each remains unknown. Using the New Zealand hybrid mouse model system of SLE, we present here a new fetal liver chimera-based system in which we can segregate effects of immune system genes from that of sex hormones. We show that female hematopoietic cells express an intrinsic capacity to drive lupus-like disease in both male and female recipient mice, suggesting that this capacity is hormone independent. Particularly, only chimeric mice with a female hematopoietic system showed significantly increased numbers of germinal center B cells, memory B cells and plasma cells followed by a spontaneous loss of tolerance to nuclear components and hence elevated serum antinuclear autoantibodies. A protective effect of testosterone was noted with regard to disease onset, but not disease incidence. Thus, genetic factors encoded within the female hematopoietic system can effectively drive lupus-like disease even in male recipients.

INTRODUCTION

Autoimmune diseases such as systemic lupus erythematosus (SLE) have a strong female bias. A female predominance is also observed in the New Zealand hybrid mouse model of SLE ((NZB × NZW)F1), where 100% of female mice but <40% of male mice develop end-stage renal disease within 1 year of age. Lupus-like disease in (NZB × NZW)F1 mice is characterized by elevated antinuclear autoantibodies (ANA), IgG-immune complex deposition and complement fixation in the kidney glomeruli, and glomerulonephritis resembling the human disorder. The disease is generally believed to be mediated by immune system defects as shown in bone marrow (BM) transfer studies.

Levels of sex hormones or differences in sex-linked gene expression patterns are proven explanations for the pronounced sex difference observed for (NZB × NZW)F1 lupus-like disease. In this regard, prepubertal hormonal manipulation studies have shown a protective effect of testosterone and exacerbating effect of estrogens. In addition, exposure to sex hormones during embryogenesis can affect autoimmune development in adult mice.

Genetic overexpression of X-linked genes, as seen in mice carrying the Yaa lupus susceptibility locus, has also been strongly associated with disease development. Particularly, a link between copies of Tlr7 and the development of ANA has been demonstrated although other genes expressed on the X chromosome probably also have a role, as demonstrated in toll-like receptor 7 (TLR7)-deficient male B6.Nbaa2(Yaa) congenic lupus-prone mice. Also supporting an effect of the X chromosome are data showing a correlation between pristane-induced lupus-like disease and X-chromosome dosage in castrated Sry-transgenic male mice and the accelerated spontaneous development of lupus in XX versus XY NZM2328 mice.

Type I interferons (IFNα) have a crucial role in SLE and lupus-like disease development. In (NZB × NZW)F1 mice, elevation of the levels of IFNα increases autoantibody production and accelerates renal disease onset. IFNα can be produced by many cell subsets, but most noticeably by plasmacytoid dendritic cells in response to a variety of stimuli targeting intracellular TLR 7, 8 and 9 and cytoplasmic DNA sensors such as Aim2, DAI/ZBP1, Lrhrf1 and IFI16 (Ifi204). IFNα is known to affect T cells, as well as B cells, although whether one or both mechanisms are involved in IFNα-driven lupus-like disease is still unknown.

In this study, we investigated whether female hematopoietic stem and progenitor cells were capable of driving autoimmunity in the presence of male and/or female sex hormones. Using a mixed sex chimera system, we found that female hematopoietic cells (HCS) could drive the development of lupus-like renal disease, elevated levels of germinal center B cells, memory B cells and plasma cells, and increased ANA in all recipients, regardless of sex hormone levels. In addition, mice receiving female HCs expressed elevated levels of serum IFNα before the generation of ANA and the onset of renal disease. Male recipients of female HCs exhibited a delay in the onset of disease as compared with female recipients, suggesting that the protective effect of testosterone affected early events in disease propagation only. Thus, it is believed that female hematopoietic cells in the absence of sex hormones, after the generation of ANA, can effectively drive lupus-like disease even in male recipients.
Results

Female BM cells transfer renal disease into male and female recipients with a higher incidence and faster kinetic than male BM cells

Estrogens are known to promote lupus-like disease development in (NZB x NZW)F1 mice, while testosterone has been found to protect against the disease. In addition, X-chromosome dosage has been found to affect disease development in other mouse models of SLE.17,27 As sex hormones affect the immune system, distinguishing the effect of hormones from the effect of genes has been challenging. We asked if female HCs from (NZB x NZW)F1 mice could transfer disease into hormonally intact, lethally irradiated, age-matched male recipients. The major male antigen H–Y is not presented by H2d and H2z, allowing such reconstitution to occur without rejection.30 To avoid potential effects of pubertal sex hormones, we performed the experiments using 4-week-old, prepubertal mice. Female HCs were capable of driving lupus-like renal disease in 100% of recipient mice by 31 weeks after transfer, regardless of the sex of the recipient mouse (Figure 1a). In contrast, within the same time frame, male HCs transferred disease into 57% and 25% of female and male recipients, respectively (Figure 1a, not statistically different within the same time frame). Male BM cells transferred disease into 76% of unmanipulated male and female recipients, as BM samples from 4-week-old unmanipulated (NZB x NZW)F1 mice showed no signs of graft rejection. 30 To avoid potential effects of pubertal sex hormones, we performed the experiments using 4-week-old, prepubertal mice. Female HCs were capable of driving lupus-like renal disease in 100% of recipient mice by 31 weeks after transfer, regardless of the sex of the recipient mouse (Figure 1a). In contrast, within the same time frame, male HCs transferred disease into 57% and 25% of female and male recipients, respectively (Figure 1a, not statistically different within the same time frame). Even when kept until 1 year of age (48 weeks after transfer), only ~85% of recipient mice accepting male HCs developed renal disease (P = 0.001). Irradiation and reconstitution itself accelerated end-stage lupus-like disease development in all recipient mice regardless of sex, although the characteristic difference between control male-into-male and female-into-female remained statistically significant (P = 0.001). Disease onset was similar in male and female mice receiving female BM cells, while male-into-male BM chimera mice started developing disease slightly later than male-into-female BM chimera mice (Figure 1a, not statistically significant).

The differential disease development was not driven by differences within the stem cell and progenitor cell compartment of male and female recipients, as BM samples from 4-week-old unmanipulated male and female (NZB x NZW)F1 mice showed equivalent levels of hematopoietic stem cells, common myeloid progenitors, common lymphoid progenitors, granulocyte–macrophage progenitors and megakaryocyte–erythocyte progenitors (Figure 1b). In addition, all mice analyzed had grafted successfully, as noted by the relative expression of Xist and Uty transcripts in peripheral blood mononuclear cell (PBMC) fractions from mice receiving female or male HCs (Figure 1c). Moreover, recipient mice continued to express sex hormones at levels equivalent to unmanipulated mice, as determined by serum levels of estradiol and testosterone (Figures 1d and e). Thus, female HCs from prepubertal 4-week-old (NZB x NZW)F1 mice transferred

Figure 1. Female prepubertal BM cells transfer lupus-like disease in a hormone-independent manner. Four-week-old (NZB x NZW)F1 male and female mice were lethally irradiated and reconstituted with male or female BM cells from age-matched mice. (a) Mice were followed for the development of renal disease by detection of proteinuria every 2 weeks. Mice with severe proteinuria (>100 mg/dL on two consecutive readings) were considered positive. All mice were killed 48 weeks after transfer, regardless of disease stage. Female-into-female (open square, n = 6); female-into-male (light gray triangle, n = 7); male-into-male (filled circle, n = 6); male-into-female (dark gray triangle, n = 7). (b) BM cells were isolated from 4-week-old unmanipulated (NZB x NZW)F1 mice (n = 5 for both males and females) and the proportions of hematopoietic stem cells and progenitor cell subsets were determined by flow cytometry. (c) PBMCs were isolated from BM chimera mice 18 weeks after transfer (n = 2 of each). Total RNA was isolated and cDNA generated. The levels of Xist and Uty transcripts were normalized to the levels of β-2-microglobulin and the % was calculated relative to the levels in control female-into-female (100% Xist) or male-into-male (100% Uty) BM chimera mice. (d) and (e) Serum was isolated from BM chimera mice 18 weeks after transfer and levels of testosterone (d) and estradiol (e) were measured by ELISA. Female recipient: n = 13 (testosterone), n = 6 (estradiol); male recipient: n = 15 (testosterone), n = 5 (estradiol); female control: n = 10 (testosterone), n = 10 (estradiol); male control: n = 6 (testosterone), n = 7 (estradiol). ***P < 0.001.
accelerated renal disease into both male and female age-matched (NZB × NZW)F1 mice independently of the recipient’s sex hormone environment.

The capacity of female HCs to transfer renal disease is present in utero

Sex hormones are produced at high levels starting at puberty. However, even in utero and during the postnatal period sex hormones are produced, and hence HCs from 4-week-old female (NZB × NZW)F1 mice could have acquired their autoimmune capacities as a result of such exposure. To test for this possibility, we generated fetal liver (FL) chimera mice. FL cells were isolated from male or female (NZB × NZW)F1 embryos at days E13.5–E14.5 and transferred into lethally irradiated 4-week-old prepubertal male or female (NZB × NZW)F1 mice. Mice were followed for the development of proteinuria until 32 weeks after transfer. Diagnosis of disease was confirmed by detection of elevated colocalized IgG-immune complex deposition and complement fixation in kidney glomeruli of chimeric mice that had received female FL cells (Figure 2d).

Similar to the experiments involving BM cell transfer from 4-week-old donors, female FL cells induced a rapid onset of disease in 100% of recipient mice, while male FL cells induced less disease and significantly delayed disease onset (Figures 2a and b, P < 0.001). However, disease occurred somewhat later in male, versus female, recipients of female FL cells (Figure 2b, P < 0.01). Again, we did not find this to be a result of differences among the transferred HCs, as analyses of FL cells from male and female (NZB × NZW)F1 embryos showed no differences in the distribution of cell subsets (Figure 2c). Similar to the BM chimeric mice, serum levels of sex hormones in FL chimeric recipient mice were comparable to that of unmanipulated male and female (NZB × NZW)F1 mice (data not shown).

Reconstitution with female FL cells specifically affects levels of postactivation B-cell subsets

Lupus is a B-cell and autoantibody-mediated disorder. We tested if B-cell numbers and subset distribution were different between the four groups of FL chimera mice. Gating strategies are depicted in Figures 3a–d. In spleens, neither the total numbers of B cells (CD19⁺) nor of marginal zone B cells (CD19⁺CD21hiCD23loIgMhiIgDlo) were significantly different between the various FL chimera mice (Figures 3e and g). However, mice that had received female FL cells displayed overall increased levels of follicular mature B cells (CD19⁺CD21loIgMloIgDlo) (Figure 3f), regardless of the sex of the recipient. Even more strikingly, the number of germinal center B cells (CD19⁺CD21hiPNA⁺IgMloIgDlo) were significantly different between the various FL chimera mice (Figures 3e and g). However, mice that had received female FL cells displayed overall increased levels of follicular mature B cells (CD19⁺CD21loIgMloIgDlo) (Figure 3f), regardless of the sex of the recipient.
Autoantibody production is driven by female HCs and not affected by the presence of male sex hormone

Female (NZB × NZW)F1 mice develop hypergammaglobulinemia at early ages followed by a specific loss of tolerance to nuclear autoantigens. In contrast, the levels of anti-chromatin IgG, anti-histone IgG and anti-dsDNA IgG were all significantly elevated in chimera mice that had received female FL cells as compared with those mice receiving male FL cells (Figures 4d–f, P < 0.05–0.001), suggesting that mice that had received female HCs displayed a specific loss of tolerance to nuclear antigens.

Levels of serum IFNα, but not BAFF, are elevated in chimera mice receiving female FL cells

The data above suggest that loss of tolerance to nuclear antigens and renal disease is accelerated by female HCs. Possibly explanations for this observation include an increased capacity of female-derived B cells to differentiate into autoantibody-producing cells or increased levels of B-cell-differentiating signals secreted by female-derived non-B-cell HCs. To test the latter idea, we examined FL chimera mice for levels of serum B-cell-activating...
factor (BAFF) at 16 weeks after irradiation and reconstitution, before the onset of renal disease. BAFF is known to be involved in B-cell survival and differentiation and has previously been found to be associated with lupus in several mouse models.33–37 FL chimera mice that had received female HCs did not express elevated levels of BAFF (Figure 5a). In fact, male FL chimera mice were found to express higher levels of BAFF than female FL chimera mice, regardless of whether these had received male or female FL cells ($P < 0.05$).

IFN $\alpha$ is also known to influence B-cell differentiation38 and is capable of driving disease development in (NZB × NZW)F1 mice and related strains.20,39–41 We tested serum levels of IFN $\alpha$ in FL chimera mice 12 weeks after irradiation and reconstitution, before the onset of renal disease. Mice reconstituted with female FL cells displayed higher levels of serum IFN $\alpha$ than mice reconstituted with male FL cells (Figure 5b, female versus male donor: $P < 0.05$). Furthermore, levels of serum IFN $\alpha$ at 12 weeks after transfer correlated statistically with levels of serum anti-chromatin IgG ($P < 0.001$), anti-histones IgG ($P < 0.05$) and anti-dsDNA IgG ($P < 0.05$) measured 20 weeks after transfer (Figures 5c–e). Serum IFN $\alpha$ levels measured 12 weeks after reconstitution also trended toward a negative correlation with the onset of renal disease in all FL chimeras (Figure 5f, $P = 0.1$).

**DISCUSSION**

Although tremendous amounts of research have gone into determining the etiology of SLE, the underlying mechanism(s) driving disease initiation and/or progression are still poorly defined. We and others have previously shown that manipulation of sex hormone production from puberty significantly alters the development of renal disease.3,6–9 Specifically, castration of male lupus-prone (NZB × NZW)F1 mice was found to remove the protective effect of testosterone, resulting in disease development equivalent to that of female unmanipulated mice.3,6 Conversely, ovariectomy of female (NZB × NZW)F1 mice before puberty fails to alter disease kinetics,6,8 suggesting that after the immune system is established in female mice around 2–3 weeks of age, estrogens are not crucial for disease progression. Here we have shown that female HCs are capable of driving lupus-like disease in hormonally intact, lethally irradiated male (NZB × NZW)F1 FL recipient mice. Thus, even in the presence of testosterone, female HCs from lupus-prone (NZB × NZW)F1 mice cannot be held back and proceed to generate autoreactive B cells, followed by IgG-immune complex deposition, glomerulonephritis and renal failure.

Pre-B-cell lines from FLs of lupus-prone (NZB × NZW)F1 mice have previously been shown to possess intrinsic autoimmune...
competencies when compared with pre-B-cell lines established from non-lupus-prone strains; however, whether these cell lines were of a male or female origin was not reported and remains unknown. As the immune system of FL chimera mice originates from transferred stem cells and progenitor cells, rather than more mature lymphocytes, our data suggest that the defect is genetically encoded. A major player in B-cell development and differentiation is Bruton’s tyrosine kinase (Btk) encoded by the X chromosome. Although not much is known about Btk levels and activity in lupus, a recent study reported amelioration of end-stage lupus-like disease in older female (NZB/C2 NZW)\textsubscript{F1} mice treated with a Btk inhibitor. The study did not investigate if males were equally susceptible, and thus any sex-driven abnormality remains to be identified. Another candidate gene is Cd40l, also encoded by the X chromosome. CD40L is essential for T-cell-dependent B-cell activation and has been assigned an essential role in (NZB \times NZW)\textsubscript{F1} lupus-like disease development, however, whether CD40L-mediated B-cell activation is differentially active in male and female recipients remains unknown.

IFN\textalpha is recognized as a key cytokine in SLE and mouse lupus-like disease. However, whether these cell lines were of a male or female origin was not reported and remains unknown. As the immune system of FL chimera mice originates from transferred stem cells and progenitor cells, rather than more mature lymphocytes, our data suggest that the defect is genetically encoded. A major player in B-cell development and differentiation is Bruton’s tyrosine kinase (Btk) encoded by the X chromosome. Although not much is known about Btk levels and activity in lupus, a recent study reported amelioration of end-stage lupus-like disease in older female (NZB/C2 NZW)\textsubscript{F1} mice treated with a Btk inhibitor. The study did not investigate if males were equally susceptible, and thus any sex-driven abnormality remains to be identified. Another candidate gene is Cd40l, also encoded by the X chromosome. CD40L is essential for T-cell-dependent B-cell activation and has been assigned an essential role in (NZB \times NZW)\textsubscript{F1} lupus-like disease development, however, whether CD40L-mediated B-cell activation is differentially active in male and female recipients remains unknown.

IFN\textalpha is recognized as a key cytokine in SLE and mouse lupus-like disease. However, whether these cell lines were of a male or female origin was not reported and remains unknown. As the immune system of FL chimera mice originates from transferred stem cells and progenitor cells, rather than more mature lymphocytes, our data suggest that the defect is genetically encoded. A major player in B-cell development and differentiation is Bruton’s tyrosine kinase (Btk) encoded by the X chromosome. Although not much is known about Btk levels and activity in lupus, a recent study reported amelioration of end-stage lupus-like disease in older female (NZB/C2 NZW)\textsubscript{F1} mice treated with a Btk inhibitor. The study did not investigate if males were equally susceptible, and thus any sex-driven abnormality remains to be identified. Another candidate gene is Cd40l, also encoded by the X chromosome. CD40L is essential for T-cell-dependent B-cell activation and has been assigned an essential role in (NZB \times NZW)\textsubscript{F1} lupus-like disease development, however, whether CD40L-mediated B-cell activation is differentially active in male and female recipients remains unknown.
depend on TLR7 signaling and are found predominantly in young autoimmune females and (2) TLR7 agonist stimulation of human PBMCs resulted in significantly more IFNγ production from female than male cells.

As estrogen receptor α expression is required for disease development in female (NZB × NZW)F1 mice, it is interesting to note that IFNα induces transcription of the Esr1 gene and subsequent expression of the estrogen receptor α. Oppositely, estrogen treatment has been suggested to drive dendritic cell activation and enhance IFNα production upon at least TLR9 ligation. Whether male and female HCs respond equally to estrogens has been evaluated both in vivo and in vitro in (NZB × NZW)F1 and non-autoimmune mice, showing no significant differences.

On the basis of these observations, we do not believe that the disease-promoting effect of female HCs is because of increased responsiveness of female cells to low levels of estrogens present in male recipients, although further experiments are needed to firmly rule out this possibility.

So what about testosterone and its well-established protective effect? We have recently described the presence of a population of testosterone-induced immunosuppressive myeloid cells (Gr1<sup>high</sup>CD11b<sup>+</sup>) in male (NZB × NZW)F1 mice. These cells have the capacity to directly suppress B-cell differentiation in vitro, while depletion in vivo promotes autoantibody production in male mice. In our chimera system, male recipients experienced a later onset of disease, although autoantibody production seemed to be only marginally lower in male versus female recipients. Comparing intact and castrated male recipient mice after reconstitution with female FL-derived HCs confirmed that the percentage of Gr1<sup>high</sup>CD11b<sup>+</sup> cells was in fact reduced in castrated male FL recipients (P<0.05); however, autoantibody levels were unchanged (unpublished results). On the basis of these observations, we propose that testosterone may act by promoting the development of immunosuppressive Gr1<sup>high</sup>CD11b<sup>+</sup> cells capable of delaying, but not inhibiting, disease development. The presence of these cells is subsequently enough to control intrinsic disease-promoting signals in male HCs but not in female HCs, the latter promoting elevated IFNα production, B-cell differentiation, ANA production and fatal renal disease. Taken together, we have found that (NZB × NZW)F1 female HCs have an intrinsic ability to drive autoimmune lupus-like disease, regardless of the hormonal environment of the host. This strongly implicates genetic rather than hormonal factors as the underlying mechanism driving the increased incidence of autoimmunity in female recipients as compared with male recipients.

**MATERIALS AND METHODS**

Mice and cells

Three-week-old male and female (NZB × NZW)F1 mice were obtained from The Jackson Laboratory and kept in a specific pathogen-free environment at National Jewish Health (Denver, CO, USA). All mouse experiments were approved by the local IACUC committee. BM chimeric mice were generated by lethal irradiation (1000 rad) using a Cs<sup>137</sup> irradiator of 4-week-old male or female (NZB × NZW)F1 mice. Male and female prepubertal (NZB × NZW)F1 mice and 5-week-old control NZW mice were given acidified saline. For detection of total IgG and IgM, serum was diluted 1:50,000–1:20,000 in serum diluent (sterile filtered 0.5% bovine γ-globulin, 5% gelatin, 0.05% Tween in 1 × phosphate-buffered saline). For detection of ANAs, serum was diluted 1:300. Levels of anti-β2-glycoprotein I, anti-ribosomal P protein and anti-dsDNA IgG autoantibodies were measured as described previously.

All reactions were developed using 10 mg ml<sup>−1</sup> 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in McIlVain’s buffer (0.09 μM Na<sub>2</sub>PO<sub>4</sub>, 0.06 μM citric acid, pH 4.6). Anti-dsDNA IgG levels were determined using the manufacturer’s protocol (Alpha Diagnostic International Inc., San Antonio, TX, USA).

**Flow cytometry**

Flow cytometry was performed using a Cyan Flow cytometer ADP (Beckman Coulter, Indianapolis, IN, USA) and all analyses were carried out using FlowJo version 9.5.2 (TreeStar Inc., Ashland, OR, USA). Antibodies with the following specificities were used for all analyses: CD11b, CD11c, CD19, CD21, CD23, CD38, CD40, B220 (CD45R), CD138, F4/80, Gr1 (Ly6C/6G), IgM and IgD (all from eBiosciences, San Diego, CA, USA). Peanut agglutinin (PNA) was obtained from Vector Laboratories Inc. (Burlingame, CA, USA).

**Immunofluorescence staining**

IgG deposition and complement factor 3 fixation was measured by immunofluorescence staining. Briefly, half kidneys were quick-frozen in OCT and 5 µm sections were prepared. Sections were stained using Texas Red-conjugated anti-mouse IgG (Invitrogen, Grand Island, NY, USA) and fluorescein isothiocyanate-conjugated anti-mouse complement factor 3-specific antibodies (ICL Inc., Portland, OR, USA). Images were collected using an HC Plan Apo 20×/0.7NA objective lens on a Leica DMR upright microscope (Leica Microsystems, Buffalo Grove, IL, USA) equipped with a Retiga EXi Cooled CCD Camera (QImaging, Surrey, BC, Canada).

**Statistical analyses**

All statistical analyses were carried out using GraphPad Prism v. 5.04 (GraphPad Inc., La Jolla, CA, USA). Analyses of cumulative incidence were carried out using a log-rank test (Mantel–Cox test). Comparisons of average time of onset, cellular proportions and serum cytokine levels between two groups were carried out using a two-tailed non-parametric Mann–Whitney test. P-values <0.05 were considered statistically significant.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.
ACKNOWLEDGEMENTS

We thank Shirley Solus and Joshua Loomis for their help with flow cytometric experiments. This study was supported in part by the Denver Autoimmune Center of Excellence and USPHS Grants AI18785 and AI22295 (to PM) and by R21AI083804 (to TNJ).

REFERENCES

1. Whitacre CC. Sex differences in autoimmune disease. Nat Immunol 2001; 2: 777–780.
2. Mellors RC. Autoimmune and immunoproliferative diseases of NZB/B1 mice and hybrids. Int Rev Exp Pathol 1966; 5: 217–252.
3. Gubbev Bopp MR, Jorgensen TN, Kotzin BL. Identification of candidate genes that influence sex hormone-dependent disease phenotypes in mouse lupus. Genes Immun 2006; 9: 47–56.
4. Helyer BJ, Howie JB. Renal disease associated with sex hormone treatment in NZB/NZW F1 mice. Nature 1963; 197: 197.
5. Morton JT, Siegel BV, Rapoport R. Suppression of glomerulonephritis in lethally irradiated DBA/2 recipients by NZB bone marrow cells. Transplantation 1975; 19: 464–469.
6. Roussinov JR, Talal N, Greenspan JS, Goodman JR, Sitienei PK. Effect of castration and sex hormone treatment on survival, anti- nuclear acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. J Exp Med 1978; 147: 1568–1583.
7. Roussinov J, Talal N, Sitienei PK, Sadakian JA. Sex hormone modulation of auto- immune manifestations in NZB/NZW mice. J Immunol 1977; 12: 1162–1169.
8. Roussinov JR, Papoian R, Talal N. Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. J Clin Invest 1977; 59: 1066–1070.
9. Wu WM, Lin BF, Su YC, Suen JL, Chiang BL. Tampoxen decreases renal inflammation and alleviates disease severity in autoimmune NZB/W F1 mice. Scand J Immunol 2000; 52: 393–400.
10. Talal N, Ahmed SA, Dauphine M. Hormonal approaches to immunotherapy of autoimmune disease. Ann NY Acad Sci 1986; 475: 320–328.
11. Murphy ED, Roths JBA. Y chromosome associated factor in strain BXSB producing accelerated autoimmune and lupus nephropathy. Arthritis Rheum 1979; 22: 1188–1194.
12. Subramanian S, Tus K, Li QZ, Wang A, Tian XH, Zhou J et al. Tlr7 translocation accelerates systemic autoimmunity in murine lupus. Proc Natl Acad Sci USA 2000; 103: 9970–9975.
13. Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoimmune B cell responses to RNA- related antigens due to TLR7 gene duplic.ation. Science 2006; 312: 1669–1672.
14. Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, Ward JM et al. Control of toll-like receptor 7 expression is essential to restrict autoimmune and dendritic cell proliferation. Immunity 2007; 27: 801–810.
15. Rubtsova AV, Rubtsova K, Kappler J, Marrack P. TLR7 drives accumulation of ABCs and transdifferentiation of dendritic cells in autoimmune-prone mice. J Immunol Res 2013; 55: 210–216.
16. Santiago-Raber ML, Dunand-Sauthier I, Wu T, Li QZ, Uematsu S, Akira S. TLR7/8 are cytosolic DNA sensor and an activator of innate immune response. J Exp Med 2010; 210: 283–299.
17. Takaoka A, Wang Z, Choi MK, Yanai H, Negishi H, Ban T et al. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 2007; 448: 501–505.
18. Blanco P, Palacua AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science 2001; 294: 1540–1543.
19. Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. Arthritis Rheum 2005; 52: 1491–1503.
20. Mathian A, Weinberg A, Gallegos M, Banchereau J, Koutouzov S. IFN-alpha induces early lethal lupus in preimmune (New Zealand Black × New Zealand White) F1 but not in BALB/c mice. J Immunol 2005; 174: 2499–2506.
21. Takaoka A, Wang Z, Choi MK, Yanai H, Negishi H, Ban T et al. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 2007; 448: 501–505.
22. Burckstummer T, Baumann C, Bluml S, Dixit E, Dumburger G, Jahn H et al. An orthogonal prothrombin-cleaving thrombin identifies AM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol 2009; 10: 266–272.
23. Yang P, An H, Liu X, Wen M, Zheng Y, Rui Y et al. The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. Nat Immunol 2010; 11: 487–494.
24. Unterholzer L, Keating SE, Baran M, Horan KA, Jensen SB, Sharma S et al. IFIT6 is an innate immune sensor for intracellular DNA. Nat Immunol 2010; 11: 997–1004.
different requirements for IRAK1/4 kinase activity across human cell types. J Immunol 2011; 186: 1279–1288.

51 Chauhan SK, Singh VV, Rai R, Rai M, Rai G. Distinct autoantibody profiles in systemic lupus erythematosus patients are selectively associated with TLR7 and TLR9 upregulation. J Clin Immunol 2013; 33: 954–964.

52 Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S et al. The nature of the principal type 1 interferon-producing cells in human blood. Science 1999; 284: 1835–1837.

53 Clingan JM, Matloubian MB. Cell-intrinsic TLR7 signaling is required for optimal B cell responses during chronic viral infection. J Immunol 2013; 191: 810–818.

54 Berghofer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H. TLR7 ligands induce higher IFN-alpha production in females. J Immunol 2006; 177: 2088–2096.

55 Wang JP, Zhang L, Madera RF, Woda M, Libraty DH. Plasmacytoid dendritic cell interferon-alpha production to R-848 stimulation is decreased in male infants. BMC Immunol 2012; 13: 35.

56 Panchanathan R, Shen H, Zhang X, Ho SM, Choubey D. Mutually positive regulatory feedback loop between interferons and estrogen receptor-alpha in mice: implications for sex bias in autoimmunity. PLoS One 2010; 5: e10868.

57 Bynote KK, Hackenberg JM, Korach KS, Lubahn DB, Lane PH, Gould KA. Estrogen receptor-alpha deficiency attenuates autoimmune disease in [NZB × NZW]F1 mice. Genes Immun 2008; 9: 137–152.

58 Seillet C, Rouquie N, Foulon E, Douin-Echinard V, Krust A, Chambon P et al. Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor alpha. J Immunol 2013; 190: 5459–5470.

59 Li X, Xu Y, Ma L, Sun L, Fu G, Hou Y. 17Beta-estradiol enhances the response of plasmacytoid dendritic cell to CpG. PLoS One 2009; 4: e8412.

60 Matsubara S, Swasey CH, Loader JE, Dakhama A, Joetham A, Ohnishi H et al. Estradiol determines sex differences in airway responsiveness after allergen exposure. Am J Respir Cell Mol Biol 2008; 38: 501–508.

61 Aronica SM, Dozier A, Fantl P, Nazareth M. Altered bone marrow cell sensitivity in the lupus-prone NZB/W mouse: regulation of CFU-GM colony formation by estrogen, tamoxifen and thrombopoietin. Lupus 2000; 9: 271–277.

62 Trigunaite A, Khan A, Der E, Song A, Vairakti S, Jorgensen TN. Gr1 CD11b cells suppress B cell differentiation and lupus-like disease in lupus-prone male mice. Arthritis Rheum 2013; 65: 2392–2402.