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The Lupus-Prone NZM2410/NZW Strain–Derived Sle1b Sublocus Alters the Germinal Center Checkpoint in Female Mice in a B Cell–Intrinsic Manner

Eric B. Wong,*1 Tahsin N. Khan,*1 Chandra Mohan,† and Ziaur S. M. Rahman*1

C57BL/6 (B6) mice carrying the Sle1b sublocus (named B6.Sle1b), which harbors the lupus-associated NZM2410/NZW SLAM family genes, produce antinuclear Abs (ANAs). However, the role and mechanism(s) involved in the alteration of the germinal center (GC) tolerance checkpoint in the development of ANAs in these mice is not defined. In this study, we show significantly higher spontaneously formed GCs (Spt-GCs) in B6.Sle1b female mice compared with B6 controls. We also found a significant increase in CD4+CXCR5PD-1hi spontaneously activated follicular Th cells in B6.Sle1b female mice. Compared with B6 controls, B6.Sle1b female mice had increased numbers of proliferating B cells predominantly located in Spt-GCs. The elevated Spt-GCs in B6.Sle1b mice were strongly associated with increased ANA-specific Ab-forming cells and ANA titers. The increased numbers of Spt-GCs and spontaneously activated follicular Th cells in B6.Sle1b mice were not the result of a generalized defect in B cells expressing Sle1b. Consistent with the elevated spontaneous response in B6.Sle1b mice, the attenuated GC response characteristic of DNA and p-azophenylarsonate reactive B cells from Ig VH knock-in mice (termed HKIR) were relieved in adoptively transferred recipients in the presence of Sle1b. Finally, by generating mixed bone marrow chimeras, we showed that the effect of Sle1b on Spt-GC, follicular Th cell, and autoantibody responses in B6.Sle1b mice was B cell autonomous. These data indicate that the NZM2410/NZW-derived Sle1b sublocus in conjunction with the female sex primarily affects B cells, leading to the alteration of the GC tolerance checkpoint and the generation of ANA-specific Ab-forming cells. The Journal of Immunology, 2012, 189: 000–000.

The lupus-prone New Zealand Black/New Zealand White (NZB/NZW)–derived NZM2410 mouse strain develops a disease phenotype that closely resembles human systemic lupus erythematosus (SLE). Three major genomic intervals (Sle1, Sle2, and Sle3) are responsible for systemic autoimmune disease susceptibility in NZM2410 mice (1–3). B6 mice congenic for the Sle1 locus develop high titers of IgG autoantibodies against chromatin (4) and generate T cells specific for histone (5), implicating Sle1 in the loss of tolerance that leads to the development of antinuclear Abs (ANAs). Genetic recombination of the Sle1 locus has further dissected the locus into four subloci termed Sle1a, Sle1FcR, Sle1b, and Sle1c (6, 7). B6 mice congenic for each sublocus display partial autoimmune phenotypes with B6.Sle1b mice exhibiting sex-biased and highly penetrant ANA production (6). Sle1b results in altered functions in both T and B cells (5, 7–9). Resting B cells from B6.Sle1b mice appear to be more readily activated and have an enhanced ability to present Ag compared with B6 controls from B6 mice (10). T cells from B6.Sle1b exhibit a higher Ca2+ flux response after TCR stimulation (7). In addition, a larger percentage of CD4+ T cells from B6.Sle1b are CD69+CD62L+CD44hi (9). Further confirmation of the importance of the Sle1b sublocus in SLE pathology is evident in B6. Sle1b mice, which also have either the Y-linked autoimmune accelerator (yaa) or lymphoproliferation (lpr) gene mutation, as they develop fatal lupus nephritis (11–13).

The Sle1b sublocus contains the SLAM (signaling lymphocyte activation molecule) family (Slamf) genes derived from the lupus-prone NZW mouse (7). The SLAMF cell surface receptors play an important role in regulating cellular and humoral immunity (14–16). Extensive polymorphisms in the Slamf genes have been demonstrated to be responsible for the loss of tolerance to nuclear Ags and for the induction of an autoimmune phenotype in B6. Sle1b mice (7). The Ly108.1 isoform of Ly108/Slamf6 expressed in B6.Sle1b mice is thought to be one of the strongest mediators involved in the loss of early B cell tolerance, whereas Ly108.2 expression in B6 is believed to play a role in the maintenance of tolerance (8). Other studies have implicated the protective role of CD48/Slamf2 as ablation of CD48 renders B6.Sle1b mice susceptible to the development of lupus-like autoimmune disease (17). Although several candidate genes in the SLAM family in B6.Sle1b mice may contribute to the loss of tolerance resulting in autoimmune pathology, epistatic interactions between these genes most likely mediate the severity of SLE in these mice.

B cell tolerance to self-Ags (i.e., nuclear Ags) is maintained through multiple tolerance checkpoints operative centrally in the bone marrow (BM) or peripherally in the secondary lymphoid...
organs (i.e., germinal center [GC] checkpoint). B cells undergo proliferation and somatic hypermutation in GCs, which results in B cells with high and low foreign Ag reactivity and potential autoreactivity. According to the current models of B cell selection in GCs, only high-affinity B cells receive survival signals and are then positively selected for further development into class-switched, high-affinity memory B cells and long-lived Ab-forming cells (AFCs) (18–20). B cells with low Ag affinity and/or autoreactivity die via apoptosis (negative selection) (21–23). Altered regulation of positive and negative selection in the GCs in the presence of lupus-associated genes (i.e., lupus alleles of SLAM family genes) may allow autoreactive B cells to escape the GC checkpoint, which may lead to the development of autoantibody-producing memory B cells and long-lived AFCs.

Strains of mice that develop SLE-like disease spontaneously form GCs in the spleen by 1–2 mo of age (24). Autoantibodies detected in lupus patients and lupus-prone mice bind their self-Ag with high affinity, are somatically mutated and class-switched (25–31), thus suggesting a role for the GC pathway in autoantibody production. However, the role and mechanism(s) involved in the alteration of the GC checkpoint in autoantibody production in B6. Sle1b mice is unclear.

Follicular Th (T FH) cells are a subset of CD4+ T cells specialized to aid GC B cell development through B-T costimulatory molecule interactions, which include CD40L, ICOS, PD-1, and SLAM. Although a break in peripheral B cell tolerance at the GC checkpoint may allow autoreactive B cells to escape negative selection and enter circulation, T FH cells have also been shown to play a key role in contributing to the development of autoimmunity (32–35). Sanroque mice, which have a mutation in the Roguin (Rc3hl) gene, spontaneously develop GCs in the spleen and lymph nodes, and have significantly increased numbers of activated memory T cells (33, 36). These mice develop an autoimmune profile resembling that of human SLE, coincident with increased T FH cell numbers per GC and development of anti-DNA-specific Abs (33). Mice having the yaa mutation on BXSX background (BXSXyaa) also develop severe autoimmune disease and have increased T FH cell numbers (34, 37). These data indicate the critical role of T FH cells in contributing to autoimmune pathology in several SLE mouse models. However, the role of T FH cells in the formation of spontaneous GCs (Spt-GCs) and ANA production in B6. Sle1b mice is not clear.

In this study, we performed a detailed analysis of the impact of the Sle1b subset on the loss of tolerance to nuclear Ags with an emphasis on the role in the GC pathway. We studied the formation of Spt-GCs in B6 and B6. Sle1b mice without any immunization, housing them in a pathogen-free barrier facility up to 9 mo. B6. Sle1b female mice exhibited significantly increased percentages of splenic Spt-GCs and spontaneously activated CD4+ Th cells including T FH (named Spt-T FH) compared with age- and sex-matched B6 controls. These elevated percentages of Spt-GCs and Spt-T FH cells in B6. Sle1b female mice were strongly correlated with increased numbers of dsDNA, histone, and nucleosome-specific AFCs of both IgM and IgG isotypes. These mice also exhibited high titers of serum ANA-specific IgG2 Abs. These data suggest that the peripheral tolerance checkpoint that controls the formation of Spt-GCs and Spt-T FH cell numbers is altered by the presence of Sle1b. This effect is significant in female but not in male mice, indicating a role for sex on Sle1b-mediated alteration of the peripheral tolerance checkpoint, which allows for the development of autoantibody-producing, long-lived AFCs.

To further study the nature of self-Ags and to determine B cell–autonomous effect of Sle1b on the alteration of the GC checkpoint, we have used an Ig V H chain knock-in mouse line termed HKIR (38, 39) that develops B cells reactive to the hapten p-azophenylarsonate (Ars) and also have high avidity for DNA and chromatin-based self-Ags. Because of their dual-reactivity with Ars, HKIR self-reactive B cells can be mobilized into the GC reaction (40, 41) where they can participate, but because of their autoreactivity, these cells are negatively regulated as characterized by the reduced anti-Ars GC response of DNA-Ars dual-reactive HKIR B cells compared with control B cells that are reactive only to Ars (40–42). Thus, this model allowed us to study the influence of Sle1b on the regulation of HKIR DNA-reactive B cells at the GC checkpoint. Using this system, we also showed that reduced anti-Ars GC response characteristic of DNA-Ars dual-reactive HKIR B cells was reversed in the presence of Sle1b, consistent with the data generated through spontaneous model showing alteration of the GC checkpoint by Sle1b. Our BM chimeric experiments further revealed that the alteration of the GC checkpoint by Sle1b was B cell intrinsic, and the effect of Sle1b on T cells appeared to be mediated by B cell defect caused by this subclass.

Materials and Methods

Mice

C57BL/6 (B6), B6.μMT, and B6.TCRβ6+/− mice were purchased from The Jackson Laboratory and then bred in-house. B6 mice congenic for the Sle1b subtype (named B6.Sle1b mice) (6, 11) and the Ig V H knock-in line HKIR were described previously (38, 43). The HKIR mice were crossed to B6.Sle1b to generate HKIR+/−Sle1b+ (named HKIR, Sle1b) mice. All mice were maintained in a pathogen-free barrier facility and were given only autoclaved food and water. Two- to 3- and 6- to 9-mo-old B6 and B6.Sle1b mice were used in experiments for studying spontaneous B and T cell activation in these mice. The mice designated for use in the SRBC response experiments were 5–6 wk old when used. All experimental procedures performed on these animals were conducted according to the guidelines of our Institutional Animal Care and Use Committee.

SRBC immunization

Five- to 6-wk-old B6 and B6.Sle1b mice were immunized (i.p.) with 200 μl 10% SRBC (Lampire, Pipersville, PA) in 1% PBS. Mouse spleens were harvested 12 d postimmunization for flow cytometric and immunohistochemical analyses. Serum samples were also collected from these mice before sacrifice to measure Ab titers.

Reagents and Abs for flow cytometry and immunohistological analysis

The following Abs were used for flow cytometric analysis of mouse splenocytes: V500-anti-B220 (RA3−6B2), PeCy7-anti-CD95 (FAS, Jo2), Alexa Fluor 700-anti-CD4 (RM4−5), FITC-Foxp3 (FJK−16s), PE-anti-PD-1 (J43), allophycocyanin-anti-Cy7-anti-CD25 (PC6), biotin-anti-CCXR5 (2G8), and V450-anti-Bcl-2 (K12−91) from BD Pharmingen (San Diego, CA); PerCP-Cy5.5-anti-CD69 (H1.253), PE-Cy5-anti-CD86 (GL1), allophycocyanin-anti-CD44 (IM7), PE-Cy5-streptavidin (SA) from BioSource (San Diego, CA); PE-Cy7-anti-CD26L (MEL−14) and PE-anti-CD80 (16−10A1) from BioLegend (San Diego, CA); and FITC−peanut lectin (agglutinin) (PNA) from Sigma-Aldrich (St. Louis, MO).

The following Abs were used for immunohistochemical analysis of the mouse spleen sections: biotin-mouse-anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA); alkaline phosphatase (AP)-SA; AP Blue substrate kit II; Vector NovoRED substrate kit (Vector Laboratories, Burlingame, CA); purified rat anti-mouse Ki67 (ImmuneKontact, Abingdon, U.K.); FITC−PNA (Sigma-Aldrich); biotin–metallophilic macroporphyrins Ab-1 (Abcam, Cambridge, MA); biotin-anti-IgD (11−26; Southern Bio-technology Associates); PE−anti-CD4 (GK1.5); FITC−GL7 (RA3−6B2) from BD Pharmingen; PE−anti-CD1 (J43), SA Alexa Fluor 633 (Molecular Probes); PE−anti-CD138; biotin-anti-BrU (Bu20a) from BioLegend (San Diego, CA); purified anti-Foxp3 (FJK−16s; biotinylated) and a biotinylated form of the anti-idiotypic mAb E4 (prepared in-house); and rabbit anti-mouse Bcl-2 (clone N-3; Santa Cruz Biotechnology, Santa Cruz, CA).

Adaptive transfer

B6 × B6.Sle1b Fl (B6.Sle1b+/−) recipient mice were immunized (i.p.) with 100 μg Ars-KLH (in alum) 1 wk before transfer (via retro-orbital i.v.)
of MACS-purified $2 \times 10^6$ splenic B cells from either HKIR or HKIR mice expressing Sle1b (HKIR,Sle1b) donor mice. These chimeric mice were then injected i.p. with 50 µg Ars-KLH in PBS immediately after cell transfer. Spleens from the recipient mice were harvested 5 d later and used for flow cytometric and immunohistological analysis.

**Generation of BM chimeric mice**

Ten- to twelve-week-old female B6,µMT (µMT) and B6.TCRβ-deficient (TCRβ−/−) mice were lethally irradiated with 10.5 Gy gamma radiation before transfer via retro-orbital i.v. injection of 7.5–10 × 10^6 T cell-depleted mixed BM cells isolated from 8- to 10-wk-old female donor mice (i.e., B6, B6.Sle1b, µMT, and TCRβ−/−). µMT recipients received either a 1:1 ratio of B6 and µMT or B6.Sle1b and µMT BM cells. TCRβ−/− recipients received either a 1:1 ratio of B6 and TCRβ−/− or B6.Sle1b and TCRβ−/− BM cells. The recipient chimeric mice were aged for 6 mo after transfer of BM cells.

**Flow cytometry**

Multicolor flow cytometric analysis was done using multiple combinations of the Abs listed earlier on cell suspensions prepared from spleens of 6- to 9-mo-old and SRBC-immunized B6 and B6.Sle1b mice, as well as from recipient B6.Sle1b−/− mice adoptively transferred with either HKIR or HKIR,Sle1b B cells. Biotinylated Abs were detected with SA-conjugated fluorochromes. Stained cells were analyzed using the BD LSRII flow cytometer. Data were analyzed using FlowJo software (Tree Star, San Carlos, CA). Intracellular staining for Foxp3 and Bcl-6 was performed through the use of the Foxp3 intracellular staining kit (eBioscience) following manufacturer’s directions.

**Immunohistology**

Spleen cryostat sections (5–6 µm) were prepared as described previously (44). Immunohistology was performed using the Abs listed earlier, the stained sections were analyzed using a fluorescence microscope (Leica Microsystems), and images were captured as previously described (45).

**BrdU cell proliferation experiments**

Unimmunized 6- to 9-mo-old and SRBC-immunized 5- to 6-wk-old B6 and B6.Sle1b mice were injected with BrdU (i.p., 0.6 mg/mouse; BD Bioscience, San Diego, CA) 12 h and 1–2 h before sacrifice. Immunohistological analysis of spleen sections for BrdU+ cells was performed using BrdU in

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**FIGURE 1.** Increased spontaneous activation of B cells and CD4 Th cells in B6,Sle1b female mice. Flow cytometric analysis was performed on splenocytes from 6- to 9-mo-old, sex-matched B6 and B6.Sle1b mice after staining for activated B cells using mAbs against B220, CD69, and CD86. The percentages of B220+CD69− (A) and B220+CD86− (B) activated B cells are shown in rectangular gates (left panels) and in scatter plots (right panels). Activated CD4 T cells were analyzed using Abs against CD4, CD44, CD62L, and CD69. The percentages of CD4+CD44hiCD62Llo short-lived effector and CD4+CD44hiCD62Lhi effector memory Th cells are shown in rectangular gates (C, left panel) and in scatter plots (C, middle and right panels, respectively). The percentages of CD4+CD69+ activated T cells are shown in rectangular gates (D, left panel) and in scatter plot (D, right panel). Each circle represents an individual mouse, and horizontal bars represent the mean values. Statistical analysis was performed as described in Materials and Methods. *p < 0.05, **p < 0.01, ***p < 0.001, Student t test.
ELISPOT assays

ELISPOT assays were performed as described previously (42). In brief, splenocyte suspensions from 6- to 9-mo-old B6 and B6.Sle1b mice were plated at either 1 × 10^5 cells/well in IgM- and IgG-coated or at 1 × 10^6 cells/well in dsDNA-, histone-, and nucleosome-coated multiscreen 96-well filtration plates (Millipore, Bedford, MA), diluted serially (1:2), and incubated for 6 h at 37˚C. Splenocytes from SRBC-immunized mice were plated at 1 × 10^6 cells/well. IgM-producing AFCs were detected using biotinylated anti-mouse IgM (Jackson Immunoresearch, West Grove, PA) and SA-AP (Vector Laboratories). IgG-producing AFCs were detected using AP-conjugated anti-mouse IgG (Molecular Probes, Grand Island, NY). dsDNA-, histone-, and nucleosome-specific AFCs were detected by biotinylated anti-mouse IgG1, IgG2a, IgG2b, and AP-IgG2c (Southern Biotechnology, Birmingham, AL). Biotinylated Abs were detected by SA-AP (Vector Laboratories). Plates were developed using the Vector Blue AP Substrate Kit III (Vector Laboratories). ELISPOTs were counted using a computerized imaging video system (Cellular Technology, Cleveland, OH).

ANA titers

Total serum ANA titers from 6- to 9-mo-old B6 and B6.Sle1b mice were measured in ELISA plates coated with either dsDNA, histone, or nucleosome, and detected with biotinylated anti-κ Ab (Invitrogen). Similarly, IgG subtype-specific ANA titers were measured by biotinylated IgG1, IgG2a, and IgG2b, and AP-IgG2c (Southern Biotechnology, Birmingham, AL). Biotinylated Abs were detected by SA-AP (Vector Laboratories). The plates were developed by the p-nitrophenyl phosphate (disodium salt; Thermo Fisher Scientific, Rockford, IL) substrates for AP. Serum samples were first diluted in PBS and then subsequently 2-fold serial dilution was carried out for each sample. The dilution factor for each sample was generated in a logarithmic scale via the software named Origin based on the different OD values of 0.8, 0.9, or 1.0 (at 405 nm) set for different isotype-specific ELISA. The OD values of 0.8, 0.9, or 1.0 were determined based on the linear distribution of most of the samples in any given ELISA. In this way, a dilution factor of 400 for a particular sample with an OD value of 1.0 would indicate that this particular serum sample needed to be diluted 400 times to obtain an OD value of 1.0 at 405 nm. In contrast, a serum sample with a dilution factor of 150 needed to be diluted 150 times to obtain the same OD value. Therefore, the higher the dilution factor in an individual mouse, the higher the Ab titers for that particular animal.

Statistical analysis

Statistical analysis was done using Student t test: p < 0.05, p < 0.01, and p < 0.001. Statistical significance for the correlation graphs was performed by using regression (R)ANOVA.

Results

Effects of Sle1b and sex on spontaneous activation of B cells and CD4 Th cells

Previous studies have indicated the influence of Sle1b sublocus carrying NZM2410/NZW lupus alleles of the SLAM family genes on both B cells and CD4 Th cells (9, 11, 13). However, the effects of sex on the spontaneous activation of B and T cells in B6.Sle1b mice are not defined. In this study, we performed a detailed analysis of the influence of Sle1b and sex on B cells and CD4 T cells by analyzing both male and female mice. Splenocytes obtained from 6- to 9-mo-old B6 and B6.Sle1b mice were stained with Abs against B cell activation markers CD69, CD80, and CD86. The percentages of B220^+CD69^+ (Fig. 1A, rectangular gates and scatter plot) and B220^+CD86^+ (Fig. 1B) B cells were significantly higher in B6.Sle1b female mice compared with B6. Sle1b male, B6 male, and B6 female mice. In B6 mice, although...
the percentage of B220<sup>+</sup>CD69<sup>+</sup> cells was similar between the two sexes, female mice had elevated B220<sup>+</sup>CD8<sup>+</sup> cells compared with male mice (Fig. 1A, 1B). We found no difference in the percentage of B220<sup>+</sup>CD80<sup>+</sup> B cells among the four groups of mice (data not shown).

Similar flow cytometric analysis of CD4<sup>+</sup> T cells using T cell markers, CD4, CD44, CD62L, and CD69 revealed significantly higher percentages of both CD4<sup>+</sup>CD44<sup>hi</sup>CD62L<sup>hi</sup> short-lived effector (Fig. 1C, middle panel) and CD4<sup>+</sup>CD44<sup>hi</sup>CD62L<sup>lo</sup> effector memory (Fig. 1C, right panel) Th cells in splenocytes from B6. Sle1b male compared with B6 male mice. CD4<sup>+</sup>CD44<sup>hi</sup>CD62L<sup>hi</sup> effector memory T cells appeared to be significantly increased in B6.Sle1b female compared with B6 female mice (Fig. 1C, right panel). CD4<sup>+</sup>CD44<sup>hi</sup>CD62L<sup>lo</sup> short-lived effector cells were also significantly higher in B6 female compared with male mice (Fig. 1C). We also observed that the percentage of CD4<sup>+</sup>CD69<sup>+</sup> cells in B6.Sle1b mice (male and female) was significantly increased compared with their B6 control counterparts (Fig. 1D, rectangular gates and scatter plot). These differences were also observed between males and females of each genotype (Fig. 1D).

Significantly greater Spt-GCs in B6.Sle1b mice

GCs are spontaneously formed (named Spt-GC) in lupus-prone mice (24) and human SLE patients (46, 47). Given the increased percentage of activated B cells in 6- to 9-mo-old B6.Sle1b mice, we examined whether GCs were spontaneously formed in the presence of Sle1b. Flow cytometric analysis was performed on splenocytes obtained from B6 and B6.Sle1b mice by staining with GC B cell markers B220, PNA, and Fas/CD95. The percentage of B220<sup>+</sup>PNA<sup>hi</sup>Fas<sup>hi</sup> GC B cells in B6.Sle1b male and female mice was significantly greater compared with their B6 control counterparts (Fig. 2A). Female mice of each genotype also had increased percentages of B220<sup>+</sup>PNA<sup>hi</sup>Fas<sup>hi</sup> GC B cells compared with their male counterparts (Fig. 2A). We also performed immunohistological analysis in which spleen sections obtained from 6- to 9-mo-old B6 and B6.Sle1b female mice were stained with anti-IgD (blue) and PNA (brown). Consistent with the flow cytometry data, we found increased frequencies of IgD<sup>neg</sup>PNA<sup>+</sup> GCs consisting of predominantly large GCs in B6.Sle1b female mice (Fig. 2C, lower two panels) compared with less frequent and significantly smaller GCs in B6 controls (Fig. 2C, upper two panels).

Next, to evaluate whether the increased Spt-GCs in B6.Sle1b mice resulted from a generalized defect in immune responsiveness of B cells expressing Sle1b, we immunized 5- to 6-wk-old B6 and B6.Sle1b mice with the T cell–dependent-Ag SRBC. The anti-SRBC–stimulated GC response was determined 12 d after immunization. We did not find any significant difference in the percentage of B220<sup>+</sup>PNA<sup>hi</sup>Fas<sup>hi</sup> GC B cells (Fig. 2B, rectangular gates) on SRBC challenge either between B6.Sle1b and B6 controls or between male (blue) and female (red) mice within each genotype (Fig. 2B, right panel). Whereas we observed elevated frequency and size of Spt-GCs in 6- to 9-mo-old B6.Sle1b female mice compared with age-matched B6 female controls (Fig. 2C), similar immunohistological analysis of SRBC-immunized B6 and B6.Sle1b spleens revealed no difference in the frequency and size of IgD<sup>neg</sup>PNA<sup>+</sup> GCs between B6.Sle1b and B6 female mice (Fig. 2D). These data indicate that increased number of Spt-GCs in B6. Sle1b mice was not the result of a generalized defect in the response of B cells expressing Sle1b.
Significantly greater Spt-TFH cells in B6.Sle1b mice

Given the increased percentage of spontaneously activated CD4+ T cells in B6.Sle1b mice (Fig. 1C, 1D), we next sought to evaluate whether B6.Sle1b mice had an increased percentage of Spt-TFH cells. Flow cytometric analysis of splenocytes from 6- to 9-mo-old B6 and B6.Sle1b mice using Abs against the TFH cell–specific markers CD4, CXCR5, and PD-1 exhibited significantly greater percentage of CD4+CXCR5hiPD-1hi Spt-TFH cells in B6.Sle1b mice (male and female) compared with B6 male and B6 female controls (Fig. 3A). In both B6 and B6.Sle1b mice, female mice also had significantly greater percentage of Spt-TFH cells compared with male mice (Fig. 3A).

We also examined the TFH cell response in SRBC-immunized 5- to 6-wk-old B6 and B6.Sle1b mice 12 d after immunization. We found no significant difference in the percentage of CD4+ CXCR5hiPD-1hi TFH cells between B6.Sle1b mice and B6 controls (Fig. 3B). No significant difference was observed between male (blue) and female (red) mice of each genotype at this age (Fig. 3B). These data are consistent with the anti-SRBC GC B cell response (Fig. 2B).

To study the anatomic location of CD4+CXCR5hiPD-1hi Spt-TFH cells shown in Fig. 3A, we stained spleen sections from 6- to 9-mo-old B6 and B6.Sle1b female mice with Abs against IgD (blue), CD4 (red), and PD-1 (green). We defined GCs by the absence of IgD staining in IgD+ B cell follicles because IgD is downregulated in GC B cells (Fig. 3C, dashed lines, first column). Consistent with flow cytometry data, we found significantly greater numbers of CD4+ (Fig. 3C, lower panel, second column) and PD-1hi (lower panel, third column) TFH cells in B6.Sle1b GCs, as evidenced by the overlapped yellow staining (overlay, fourth column), compared with B6 controls (Fig. 3C, top row).

We also evaluated whether increased numbers of Spt-GCs and Spt-TFH cells in B6.Sle1b mice could result from the decrease in CD4+Foxp-3+ T regulatory cells. By performing flow cytometric and immunohistological analyses, we found no difference between the two strains in the frequency of CD4+Foxp-3+ T regulatory cells within and outside of GCs (Supplemental Fig. 1).

B and T cell abnormalities occur at early age in B6.Sle1b mice

Next, we examined whether the spontaneous responses that we observed in 6- to 9-mo-old B6.Sle1b mice also occurred at earlier time points. Splenocytes from 2- to 3-mo-old unimmunized female B6.Sle1b mice and B6 controls were analyzed for spontaneous GC, TFH cell, and ANA-specific AFC responses. Although the overall responses in these mice were lower at these earlier time points compared with later time points (i.e., 6–9 mo old), we found the difference in the percentages of B220+Fas+PNAhi GC B cells in B6.Sle1b mice with sex- and age-matched B6 and B6.Sle1b mice for the percentages of GC B cells (A) and TFH cells (B). Open circles represent B6 mice, whereas the closed circles represent B6.Sle1b mice. Each circle represents an individual mouse, and horizontal bars represent the mean values. Statistical analysis was performed as described in Materials and Methods. *p < 0.05, **p < 0.01, ***p < 0.001. Student t test.

Increased Bcl-6–expressing B cells and CD4 Th cells in B6.Sle1b mice

Bcl-6 is considered to be a master transcriptional regulator for GC B cells (48–51) and TFH cells (52–54). Given the increased numbers of Spt-GCs and Spt-TFH cells in the presence of Sle1b, we evaluated whether B6.Sle1b mice harbored an increased percentage of Bcl-6–expressing B cells and CD4 Th cells. Flow cytometric analysis of splenocytes obtained from 6- to 9-mo-old B6 and B6.Sle1b mice was performed through surface staining with B220 and anti-CD4, and intracellular staining with anti-Bcl-6. The percentages of B220+Bcl-6+ B cells (Fig. 5A) and CD4+Bcl-6+ T cells (Fig. 5C) in B6.Sle1b female mice were significantly greater compared with B6.Sle1b male and B6 male and female mice. No difference was observed between B6 male and B6 female mice, and B6 male versus B6.Sle1b male mice.

Furthermore, to determine the follicular GC (F-GC; i.e., Spt-GC) versus extrafollicular (EF) localization of B220+Bcl-6+ B cells and CD4+Bcl-6+ T cells in the spleen, immunohistological analysis was performed on two consecutive spleen sections obtained from 6- to 9-mo-old B6 and B6.Sle1b female mice. One was stained with the GC B cell marker GL7 and anti–Bc1-6 (Fig. 5B). The other was stained with anti-CD4 and anti–Bcl-6 (Fig. 5D). We found elevated Bcl-6–expressing B cells (Fig. 5B) and T cells (Fig. 5D) in B6.Sle1b mice, predominantly located within GCs as evidenced by the overlapped yellow staining in the overlay images (Fig. 5B, 5D, third column) with very few such cells in the

FIGURE 4. Sle1b induces Spt-GC and T FH cell responses at early age. Flow cytometric analysis of splenocytes obtained from 2- and 3-mo-old female B6 and B6.Sle1b mice for the percentages of GC B cells (A) and T FH cells (B). Open circles represent B6 mice, whereas the closed circles represent B6.Sle1b mice. Each circle represents an individual mouse, and horizontal bars represent the mean values. Statistical analysis was performed as described in Materials and Methods. *p < 0.05, **p < 0.01, ***p < 0.001. Student t test.
EF location (i.e., T cell zone, marginal zone, and red pulp) outside of GCs.

F-GC versus EF spontaneous B cell proliferation in the presence or absence of Sle1b

Next, we evaluated spontaneous B cell proliferation in the F-GC (also designated as Spt-GC) versus EF regions in the spleens of B6 and B6.Sle1b mice. EF areas included T cell zone, marginal zone, and red pulp in the spleen. Six- to 9-mo-old B6 and B6. Sle1b female mice were injected with BrdU (0.6 mg/mouse) 12 and 1–2 h before sacrifice to obtain spleens for analysis. Spleen sections obtained from B6 and B6. Sle1b mice were either stained with GL7 (green) and anti-Bcl-6 (red) (F) or anti-CD4 (red) and anti-Bcl-6 (green) (D). Data shown in (B) and (D) were obtained from four to five female mice of each genotype. Original magnification ×200. **p < 0.01, ***p < 0.001, Student t test.

Proliferating cells within the GCs are believed to be primarily B cells. However, it is not clear whether the increased number of Spt-TFH cells in Spt-GCs of B6.Sle1b mice (Fig. 3) could result from the proliferation of CD4 T cells located within GCs. To address this issue, we performed immunohistological analysis on two consecutive spleen sections obtained from 6- to 9-mo-old B6 and B6.Sle1b female mice. One was stained with GL7 (Fig. 6D, green, first column) and anti-BrdU (Fig. 6D, red, second column), and the other was stained with anti-CD4 (Fig. 6E, green, first column) and anti-BrdU (Fig. 6E, red, second column). Although GL7+ B cells colocalized with BrdU staining as judged by the yellow overlapped staining (Fig. 6D, third column), we did not observe such overlapped yellow staining with CD4 and BrdU (Fig. 6E, third column). In addition, when we stained spleen sections from B6.Sle1b and B6 control mice with anti-IgD (blue), GL7 (green), and Ki67 (red), a cell proliferation marker, we found analogous results as in Fig. 6D showing increased numbers of Ki67+ cells in B6.Sle1b relative to B6 control mice (Fig. 6F). Together, these data indicate that BrdU+ and Ki67+ proliferating cells in GCs are B cells and not CD4 T cells. Also, these data, together with results shown in Fig. 3C, indicate that the increased number of CD4 T cells in 6- to 9-mo-old B6.Sle1b GCs is due to spontaneously activated and fully differentiated T FH cells.

Increased numbers of Spt-GCs and Spt-T FH cells in B6.Sle1b mice strongly correlate with increased number of ANA-specific AFCs

To evaluate whether increased numbers of Spt-GCs and Spt-T FH cells in B6.Sle1b mice correlated with an increased ANA-specific AFC response, we first measured the number of total IgM and IgG-producing AFCs in spleen samples from 6- to 9-mo-old B6 and B6.Sle1b mice through ELISPOT assay. The number of IgM- and IgG-producing AFCs in B6.Sle1b female mice was signifi-
cantly greater compared with B6.Sle1b female mice (Fig. 7A). Significant difference was also observed between male and female mice of each genotype in IgG-producing AFCs. Consistent with the anti-SRBC GC B cell response (Fig. 2B, 2D), we did not observe any significant difference between B6 and B6.Sle1b mice in SRBC-specific IgM and IgG AFCs (Fig. 7B) 12 d after SRBC immunization. By staining with MOMA-1 (blue), which stains for metallophilic macrophages and defines the border between follicle and marginal zone, anti-CD138 (red), a marker for AFCs, and anti-IgG (green), we showed elevated number of CD138*IgG*AFCs located both in the red pulp areas and in the bridging channels of female B6.Sle1b spleens compared with a lower number of these cells in B6 controls located primarily in the bridging channels (Fig. 7C).

To determine whether increased Spt-GCs and total IgM and IgG AFCs in B6.Sle1b mice led to elevated numbers of ANA-specific AFCs, we measured dsDNA-, histone-, and nucleosome-specific AFCs by ELISPOT assay. We found the numbers of AFCs with each of these specificities (Fig. 7D–F) were significantly greater in B6.Sle1b female mice compared with B6.Sle1b male, B6 male, and B6 female mice. No difference was observed between B6.Sle1b male and B6 male mice or B6 male versus B6 female mice in dsDNA and nucleosome-specific AFCs. We, however, observed a significant difference between B6.Sle1b male and B6 male mice in histone-specific AFCs (Fig. 7E). Interestingly, we further observed that the increased numbers of dsDNA-, histone-, and nucleosome-specific AFCs strongly correlated with elevated numbers of GC B cells in B6.Sle1b mice (Fig. 7G–I).
Increased Spt-GCs and ANA-specific AFCs in B6.Sle1b female mice led to increased serum ANA titers

To examine whether the elevated Spt-GC and AFC responses in B6.Sle1b mice correlated with increased serum ANA titers, we first measured dsDNA-, histone-, and nucleosome-specific total (IgM + IgG) Ab titers in sera collected from 6- to 9-mo-old B6 and B6.Sle1b mice (Fig. 8A–C). These titers were found to be significantly higher in B6.Sle1b mice compared with B6 controls. Histone- and nucleosome-specific Abs were also significantly increased in B6.Sle1b female compared with B6.Sle1b male mice. No difference was observed between B6 male and B6 female mice. These data were consistent with results obtained in the ANA detection assay using ANA Hep-2 substrate slides. In this assay, we found significantly higher intensity of nuclear staining with sera from B6.Sle1b female compared with background staining in B6 female mice (data not shown).

Next, we evaluated IgG subclass-specific ANA titers in 6- to 9-mo-old B6 and Sle1b mice. IgG1 ANA titers remained similar among the four groups of mice (data not shown). In contrast, B6.Sle1b female mice had significantly higher titers of IgG2c Abs specific for dsDNA, histone, and nucleosome compared with B6.Sle1b male, B6 male, and B6 female mice (Fig. 8D–F). We also observed significantly increased IgG2b Abs specific for nucleosome in B6.Sle1b female mice (Fig. 8G). Finally, the increased
IgG2c/2b ANA titers were strongly correlated with the increased number of Spt-GCs in B6. Sle1b mice (Fig. 8H–K).

Reduced GC response characteristic of Ars-DNA dual-reactive HKIR B cells was reversed in the presence of Sle1b

Data described earlier suggest that peripheral tolerance checkpoints that regulate Spt-GC formation and activation of CD4 Th cells, including T FH cells, are disrupted in the presence of Sle1b, which may contribute to the development of autoantibody-producing AFCs and memory B cells. To determine the role and nature of self-Ags in Spt-GC formation and the alteration of the GC tolerance checkpoint by the Sle1b sublocus, we used an adoptive transfer system in which we transferred Ars and DNA dual-reactive HKIR B cells into syngeneic recipients (40–42). We previously showed that dual-reactive HKIR B cells can enter GCs upon immunization with Ars-conjugated foreign Ag (i.e., Ars-KLH), but because of their autoreactivity (DNA reactivity), these cells are negatively regulated and prevented from expanding in GCs presumably by a GC tolerance checkpoint (40–42). Therefore, the HKIR model is ideal to study the effects of Sle1b on GC B cell tolerance pathways of nuclear Ag–specific B cells.

We transferred purified B cells (2 × 10^6) from B6.HKIR and B6. Sle1b (named HKIR.Sle1b) female mice into syngeneic B6 × B6. Sle1b (B6.Sle1b^+/−) mice were used as recipients to avoid allo-rejection, and B6. Sle1b^+/− mice do not display any autoimmune features, because the influence of Sle1b on lymphoid and accessory cell function is recessive (1). We used the anti-clonotypic mAb E4 to detect dual-reactive HKIR B cells as described previously (40–42). E4-specific primary AFC and GC responses were determined in spleen samples obtained on day 5 after cell transfer. Flow cytometric analysis of splenocytes revealed a significant increase in the percentage of donor-derived E4 +PNA+ GC B cells in HKIR.Sle1b→B6.Sle1b^+/− mice compared with HKIR→B6.Sle1b^+/− controls (Fig. 9A, 9B). These data were consistent with immunohistology results showing more E4+ (red) and E4 +PNA+ (yellow) cells in GCs of HKIR.Sle1b→B6.Sle1b^+/− mice (Fig. 9C, lower panel) compared with HKIR→B6.Sle1b^+/− controls (Fig. 9C, upper panel). The E4-specific short-lived AFC response to Ars was also evaluated by ELISPOT assay in the recipient mice. We did not, however, observe any significant difference between HKIR.Sle1b→B6.Sle1b^+/− and HKIR→B6.
Sle1b expression in B cells, but not in T cells, resulted in increased Spt-GCs, Tfh cells, ANA-specific AFCs, and ANAs in B6.Sle1b mice

Next, we evaluated whether phenotypic changes that were observed in T cells (i.e., activation of T cells and increased percentage of GC Tfh cells) in B6.Sle1b mice resulted from the primary effect of Sle1b on T cells or whether they were influenced by B cells expressing Sle1b. Using μMT and TCRβδ−/− mice that lack B and T cells, respectively, we generated mixed BM chimeric mice where in one group of mice (i.e., B6.Sle1b + B6.μMT marrow → B6.μMT), all reconstituted B cells expressed Sle1b, whereas in the other group (i.e., B6.Sle1b + B6.TCRβδ−/− marrow → B6.TCRβδ−/−), all reconstituted T cells expressed Sle1b in the presence of chimeric accessory compartments. We used B6 + B6.μMT BM → B6.μMT and B6 + B6.TCRβδ−/− marrow → B6. TCRβδ−/− chimeras as controls. Only female mice were used for these experiments. These mice were rested for 6 mo before analysis for the development of Spt-GCs, Tfh cells, autobody-producing AFCs, and ANAs.

Flow cytometry analysis on spleen cells obtained from these mice revealed significantly greater percentages of B220+PNA+ GC B cells (Fig. 10A) and CD4+CXCR5+PD-1+ Tfh cells (Fig. 9B) in B6.Sle1b + B6.μMT BM → B6.μMT mice compared with B6 + B6.μMT BM → B6.μMT controls. Interestingly, increased frequency of GC B cells and Tfh cells was not observed in B6.Sle1b + B6.TCRβδ−/− marrow → B6.TCRβδ−/− chimeras where all T cells expressed Sle1b, and no significant difference was observed between B6 + B6.TCRβδ−/− → B6.TCRβδ−/− and B6.Sle1b + B6.TCRβδ−/− → B6.TCRβδ−/− mice (Fig. 10A, 10B). These results were consistent with immunohistological data obtained from spleen sections showing increased frequencies of IgD−/−PNA+ GCs with predominantly large GCs in B6.Sle1b + B6.μMT → B6.μMT mice (Fig. 10C, upper right) compared with the other three groups of mice showing less frequent and significantly smaller GCs (Fig. 10C). The number of CD4+ T cells also appeared to be increased in B6.Sle1b + B6.μMT BM → B6.μMT mice compared with the other three groups (Fig. 10D).

By performing ELISPOT assay, we further observed that B6.Sle1b + B6.μMT → B6.μMT control mice had significantly higher dsDNA-, histone-, and nucleosome-specific AFCs compared with B6 + B6.μMT → B6.μMT control mice (Fig. 10E–G). In agreement with the GC B cell and Tfh cell data (Fig. 10A, 10B), we did not find any difference in ANA-specific AFCs between B6 + B6.TCRβδ−/− and B6.Sle1b + B6.TCRβδ−/− mice (Fig. 10E–G). Analogous to the AFC data, we found significantly higher dsDNA-, histone-, and nucleosome-specific Abs in B6.Sle1b + B6.μMT → B6.μMT mice compared with the other three groups of mice (Fig. 10H–J).

These data, together with results described earlier (Fig. 9), indicate that the alteration of the GC checkpoint by Sle1b is B cell autonomous and the effect of Sle1b on T cells appears to be secondary to the B cell defect.

Discussion

In this study, we determined the influence of the Sle1b genomic interval, which contains the lupus-associated Nzm2410/Nzw SLAM family genes, on the GC tolerance pathway or checkpoint that may lead to the development of elevated ANA titers. We used spontaneously developed, B cell adoptive transfer and BM transfer models to provide a comprehensive analysis of the effects of Sle1b on the GC checkpoint. In the spontaneously developed GC model, we found significantly increased percentage of GC B cells and GC Tfh cells in B6.Sle1b female mice relative to B6 female controls. Strong correlation was observed between the elevated numbers of Spt-GCs in B6.Sle1b mice and the increased numbers of dsDNA-, histone-, and nucleosome-specific AFCs, as well as high titers of serum ANAs. Using the adoptive transfer model, we found an augmented GC response of Ars-DNA dual-reactive HKIR B cells in the presence of Sle1b. In addition, by generating mixed BM chimeras, we observed that the effect of Sle1b on the alteration of the GC tolerance checkpoint was B cell autonomous and the effect of Sle1b on T cells including GC Tfh cells appeared to be secondary to the B cell defect. Together, these data suggest that genes located in Sle1b contribute to the alteration of the GC checkpoint by primarily affecting B cells leading to enhanced Spt-GCs, Spt-Tfh cells, and the generation of autobody-producing AFCs.

Given the roles of SLAM family costimulatory molecules in regulating cellular and humoral immunity (14, 15, 55, 56), and the association of SLAM family genes located in Sle1b with lupus (7–9), these genes are strong candidates for perturbing peripheral tolerance checkpoints (i.e., the GC checkpoint) in B6.Sle1b mice. Although the data presented in this article suggest that the lupus-associated SLAM family genes in Sle1b are probably responsible for the alteration of peripheral tolerance at the GC checkpoint, previous studies using the HEL Ig transgenic mouse system revealed that the Ly108.1 isofrom of the Ly108 gene in Sle1b alters early or central B cell tolerance (8). Recent studies by Keszei et al. (9) implicated the Ly108.1 gene in altering peripheral tolerance. These studies, together with the available literature, suggest that the Ly108.1 isofrom expressed by B6.Sle1b mice may alter both central and peripheral tolerance checkpoints. The polymorphisms in different members of SLAM family genes may also coordinate in altering B cell tolerance at different checkpoints.

One of the intriguing questions in studies of autoreactive B cell activation in autoimmune mouse models is where the site of a break in tolerance occurs. Whereas defects in the central tolerance checkpoint are proposed in causing autoimmunity, the development of autobody-producing, long-lived IgG+ AFCs and memory
FIGURE 10. The Sle1b sublocus primarily affects on B cells. Flow cytometric analysis was performed to evaluate the percentages of B220+Fas+PNAhi GC B cells (A) and CD4+CXCR5hiPD-1hi TFH cells (B) in splenocytes obtained from B6 + μMT → μMT, B6.Sle1b + μMT → μMT, B6 + TCRβδ−/− → TCRβδ−/−, and B6.Sle1b + TCRβδ−/− → TCRβδ−/− chimeras. Spleen sections from these mice were stained for either IgD (blue) and PNA (red) (C) or B220 (blue) and CD4 (red) (D). Original magnification ×100. The number of dsDNA- (E), histone- (F), and nucleosome-specific (G) AFCs were measured by ELISPOT assay. Total IgG dsDNA- (H), histone- (I), and nucleosome-specific (J) ANA titers were measured by ELISA. The open circles represent B6 mice, whereas the closed circles represent B6.Sle1b mice. Each circle represents an individual mouse, and horizontal bars represent the mean values. Statistical analysis was performed as described in Materials and Methods. ***p < 0.001, Student t test.
B cells in lupus cannot be explained by a defect in the central tolerance checkpoint alone because these cells are usually generated in GCs formed in peripheral lymphoid organs. Therefore, peripheral tolerance checkpoints (i.e., the GC checkpoint) may play a crucial role in the prevention of autoreactive B cells from developing into long-lived AFCs and memory B cells, which can persist for years. Numerous autoimmune mouse models, including (NZB/NZW) F1, BXSB, and sanroque, spontaneously develop GCs in the absence of an infection or immunization (24, 33, 36), and all of these mouse models develop autoantibodies against nuclear self-Ags. However, the role of a dysregulated GC tolerance pathway in autoantibody production in these mice is unclear. Our current results in B6.Sle1b mice demonstrate that increased GC formation is directly correlated to increased numbers of dsDNA-, histone-, and nucleosome-specific AFCs, as well as increased ANA titers. Direct evidence for the break in peripheral tolerance at the GC checkpoint is further demonstrated by the increase in HKIR B cells populating GCs in the presence of Sle1b. These results implicate a dysregulated GC checkpoint or pathway in ANA production and autoimmunity in B6.Sle1b mice.

Activated B cells can also develop into memory B cells and plasma cells independent of the GC outside of the follicle (57–59). The AFCs generated from this EF pathway tend to be short-lived, and the memory B cells remain largely unswitched (60). Several studies have implicated the role of the EF pathway of B cell activation in autoimmunity. Autoreactive B cells in MRL.Fas57−/− mice do not involve the GC pathway, but instead they undergo somatic hypermutation in the T cell zone (57, 58). The (NZB/NZW) F1 mice develop short-lived plasmablasts via an EF pathway that are autoreactive for dsDNA and contribute to the autoimmunity (59). Our data indicate that the Sle1 sublocus primarily affects the GC checkpoint, leading to increased numbers of ANA-specific and class-switched IgG+ AFCs and ANAs. Although a role of EF ANA-specific B cells cannot be entirely discounted in our autoimmune model, our data suggest that a major contributor to the break in peripheral tolerance leading to the production of ANA in B6.Sle1b mice is through the alteration of the GC tolerance pathway.

Tfh cells play an integral role in the formation of the GC, as well as for the maturation and development of GC B cells into long-lived AFCs and memory B cells. Several studies have implicated dysregulated Tfh cells in the development of autoimmunity (12, 33, 35–37). The autoimmune mouse models, including mice expressing two copies of TLR7 (i.e., BXSB.yaa mice) and sanroque mice have increased numbers of Tfh cells coincident with high titers of ANAs (33, 36, 37). B6.Sle1b mice have increased numbers of Spt-GC B cells along with significantly higher Tfh cell numbers. The number of Tfh cells in B6.Sle1b mice is approximately double that of B6.Sle1b yaa mice. Therefore, Sle1b appears to affect both B and T cells (5, 7–9). In support for a predominant B cell–intrinsic effect, Kumar et al. (8) have demonstrated that the presence of the Lyst08.1 isoform from the Sle1 locus renders B cells unresponsive to normal regulation at early B cell tolerance checkpoints. We previously showed that the presence of Sle1 on HKIR B cells enables them to escape GC tolerance mechanisms and allows increased participation of HKIR B cells expressing Sle1 in the GC response (42). Our current results from B cell adoptive transfer experiments (Fig. 9) showing increased participation of HKIR.Sle1b B cells in the anti-Ars GC response provide evidence for a B cell intrinsic–effect on the breach in peripheral tolerance at the GC checkpoint. These results are in agreement with our BM chimeric data (Fig. 10) showing B cell autonomous effect of Sle1b on increased Spt-GCs, Tfh cells, and ANA-specific AFCs and Abs. In contrast, studies by Keszei et al. (9) recently suggested the role for peripheral CD4+ T cells from B6.Sle1b mice in autoantibody responses by transferring purified Sle1b CD4+ T cells or CD62L− naive CD4+ T cells into bm12 mice. However, our BM chimeric data revealed no primary effect of Sle1b on T cells (Fig. 10B); rather, T cells were indirectly influenced by the expression of Sle1b in B cells, providing evidence for the requirement of B cell–intrinsic defect caused by Sle1b in the development of Spt-GCs, Tfh cell expansion, and autoantibody response.

One central theme that we observed in these studies is that only the female B6.Sle1b mice had the highest percentages of Spt-GCs, Tfh numbers, ANA-specific AFCs, and serum ANA titers. Even though male B6.Sle1b mice had higher numbers of Spt-GCs and Spt-Tfh cells compared with B6 male mice, the numbers of ANA-specific AFCs were not significantly different from B6 controls. Therefore, Sle1b appears to have the highest penetrance in female mice. To our knowledge, this is the first thorough study showing the differences in B cell/T cell activation, GC formation, and ANA-specific AFC numbers between male and female B6.Sle1b mice. Our results are in accordance with previous report by Girmaldi et al. (61) showing that estrogen alters thresholds for B cell apoptosis and activation. The importance of sex hormones in the immune system homeostasis is evident through differences between males and females in B6 mice, because female B6 mice tend to have higher spontaneously activated phenotypes. This is consistent with the findings that an overwhelming percentage of people with autoimmune diseases are female. We cannot rule out the potential role of female chromosome as the inactivation of the second X chromosome in female individuals can sometimes be incomplete, leading to dysregulated gene repression (62) that may contribute to enhanced Spt-GC, Spt-Tfh, and ANA titers observed in female mice in our studies. We are also unable to exclude the potential protective role of male hormones or chromosome in this process. It is of great interest to further dissect the role of the lupus-associated SLAM family genes in conjunction with sex hormones (i.e., estrogen) or chromosomes in breaking peripheral B cell tolerance at the GC checkpoint, leading to the development of autoantibody-producing, long-lived AFCs and memory B cells.

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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Legend:

No significant difference in regulatory T cells between B6 and B6.Sle1b mice: Splenocytes isolated from 9 month old B6 and B6.Sle1b mice were stained for CD4 and Foxp3 (A). The percentage of CD4+Foxp3+ Tregs is shown in the rectangular gates and each flow cytometry plot is a representative image for the specific group. Furthermore, splenocytes from the 6-9 month old mice were stained for follicular regulatory (TFR) T cells as defined as CD4+CXCR5hiPD-1hiFoxp3+ (B). The percentage of TFR cells is shown in the rectangular gates and each flow cytometry plot is a representative image for the specific group. (C) Spleen sections from 9 month old B6 and B6.Sle1b mice were stained for PNA (green) and anti-Foxp3 (red). High magnification (200x) representative images are displayed.