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Evidence for Genes in Addition to Tlr7 in the Yaa Translocation Linked with Acceleration of Systemic Lupus Erythematosus

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T
he BXSB strain of mice spontaneously develops an autoimmune syndrome with features of systemic lupus erythematosus (SLE) that affects males much earlier than females (1). Genetic analysis of the F1 hybrids of male BXSB mice with other lupus-prone mice demonstrated that the accelerated development of SLE in male BXSB mice is linked to the Y chromosome of the BXSB strain. Thus, the genetic abnormality present in BXSB Y chromosome has been called Yaa (Y-linked autoimmune acceleration; Ref. 2). Studies of Yaa and non-Yaa double-bone marrow-chimeric mice have demonstrated that anti-DNA autoantibodies are selectively produced by B cells bearing the Yaa mutation, and that T cells from both Yaa and non-Yaa origin efficiently promote anti-DNA autoantibody responses (3, 4). These data indicate that the Yaa defect is functionally expressed in B cells, but not in T cells. The expression of the Yaa defect in B cells has been further supported by a loss of marginal zone (MZ) B cells in spleen, which results from a defect intrinsic to Yaa-bearing B cells, independently of the development of SLE (5). More recently, the Yaa mutation was shown to be a consequence of a translocation from the telomeric end of the X chromosome and onto the Y chromosome (6, 7). Based on the presence of the gene encoding TLR7 in this translocated segment of the X chromosome, the possible role of TLR7 in the activation of autoreactive B cells (8, 9) and the development of SLE (10, 11), the Tlr7 gene duplication has been proposed to be the etiological basis for the Yaa-mediated enhancement of disease (6, 7, 12).

To determine whether the Tlr7 gene duplication is indeed responsible for the Yaa-mediated acceleration of SLE, in the present study, we generated C57BL/6 (B6) mice congenic for the Nba2 (NZB autoimmunity 2) locus (B6.Nba2) bearing the Yaa mutation revealed that the introduction of the Tlr7 null mutation on the X chromosome significantly reduced serum levels of IgG autoantibodies against DNA and ribonucleoproteins, as well as the incidence of lupus nephritis. However, the protection was not complete, because these mice still developed high titers of anti-chromatin autoantibodies and retroviral gp70-anti-gp70 immune complexes, and severe lupus nephritis, which was not the case in male B6.Nba2 mice lacking the Yaa mutation. Moreover, we found that the Tlr7 gene duplication contributed to the development of monocytesis, but not to the reduction of marginal zone B cells, which both are cellular abnormalities causally linked to the Yaa mutation. Our results indicate that the Yaa-mediated acceleration of SLE as well as various Yaa-linked cellular traits cannot be explained by the Tlr7 gene duplication alone, and suggest additional contributions from other duplicated genes in the translocated X chromosome.

Materials and Methods

Mice

The generation of B6.Nba2 Tlr7+/Yaa-congenic mice homozygous for the NZB-derived Nba2 lupus susceptibility locus (flanked by markers D1Mit47 and D1Mit61 in chromosome 1) and B6 Tlr7+/Yaa-consonomic mice have...
Expression levels of Tlr7 in splenic B cells from 2-mo-old male Tlr7+/Yaa, Tlr7−/Yaa, and Tlr7−/Yaa mice were determined using SYBR green Supermix (Bio-Rad). Results were quantified relative to a standard curve generated with serial dilutions of a reference cDNA preparation and normalized using TATA-binding protein mRNA. Note a comparable Tlr7 expression in B cells from Tlr7+/Yaa and Tlr7−/Yaa mice, which contrasted to increased expression of Tlr7 mRNA in B cells from male Tlr7+/Yaa mice (p < 0.001). Pro liferative responses of purified B cells from B6 Tlr7+/Yaa ( ), Tlr7−/Yaa ( ■ ), and Tlr7−/Yaa ( ○ ) mice after stimulation with different concentrations of imiquimod (TLR7 agonist) or CPG (TLR9 agonist). Note significantly enhanced proliferative responses by Tlr7+/Yaa B cells stimulated with 1 and 3 μg/ml of imiquimod, as compared with Tlr7−/Yaa and Tlr7−/Yaa B cells (p < 0.05). The specificity of imiquimod as a TLR7 agonist was controlled by the lack of proliferative responses of Tlr7−/− B cells ( ● ). Results (means of three mice ± SD) are expressed as cpm of [3H]Tdr incorporation.

B cells were purified from spleen by treatment with IgM anti-Thy-1.2 (AT-83) mAb in the presence of rabbit complement (Cedarlane). The purity of B cells, as documented by flow cytometric analysis, was superior to 90%. For proliferative responses of splenic B cells, 2 × 10^5 purified B cells were incubated with different concentrations of an TLR7 agonist imiquimod R837 (Invivogen) or a TLR9 agonist CpG oligonucleotide (type B CpG 1018; a gift of Dr. Eyal Raz, University of San Diego, San Diego, CA) in 0.2 ml of DMEM, 10% FCS at 37°C, and cultures were pulsed with [3H]Tdr for the final 16 h of a 3-day culture.

Quantitative RT-PCR

RNA from splenic B cells was purified with TRIzol reagents (Invitrogen). The abundance of Tlr7 mRNA was quantified by real-time RT-PCR with cDNA prepared from RNA. Tlr7 cDNA was amplified using a forward primer (5'-AGGGTATGAGGCTGGTCAGAGG ATAAAC-3'), a mutant-specific primer (5'-GCAGATATAGCCTGCTCAC TCTAGACC-3'), and a common primer (5'-ATCGGTCCTCTCTACCTCT TGTGACGAG-3'). Tlr7+/Yaa B6 and B6.Nha2 mice were established by selective crossing of Tlr7−/− B6 females with B6.Yaa or male B6.Nha2.Yaa mice. Animal studies described in the present study have been approved by the Ethical Committee for Animal Experimentation of the Faculty of Medicine, University of Geneva. Blood samples were collected by orbital sinus puncture and sera were stored at −20°C until use.

Flow cytometric analysis

Flow cytometry was performed using two- or three-color staining of PBMC and spleen cells and were analyzed with a FACS Calibur (BD Biosciences). The following Abs were used: anti-CD21 (7G6), anti-CD23 (B3B4), anti-B220 (RA3-6B2), anti-CD69 (H1.2F3), anti-CD11b (M1/70), and anti-F4/80 mAb. Staining was performed in the presence of saturating concentration of 2.4G2 anti-Fc γRIII/II mAb. The mean percentage of CD11b+ monocytes (± SD) among PBMC in 8-mo-old male B6 mice (n = 12) was 8.3 ± 2.2. Mice displaying percentages of monocytes >3 SD above the mean of B6 males (>14.9%) were considered as positive for monocytosis.

Serological assays

Serum levels of IgG autoantibodies against chromatin, dsDNA, and ribonuclear protein (RNP) were determined by ELISA. Chromatin, prepared from chick erythrocytes, and calf thymus RNP complexes (ImmuNoVision) were directly coated to ELISA plates, whereas dsDNAs were coated to ELISA plates precoated with poly-l-lysine (Sigma-Aldrich). The plates were incubated with 1/100 diluted serum samples, and the assay was developed with alkaline phosphatase-labeled goat anti-mouse IgG. Results are expressed in units per milliliter in reference to a standard curve derived from a serum pool of MRL-Fas−/− mice. Serum levels of gp70-anti-gp70 immune complexes (gp70 IC) were quantified by ELISA combined with the treatment of sera with 10% polyethylene glycol (average molecular weight, 6000), which precipitates only Ab-bound gp70, but not free gp70, as described (17). Results are expressed as micrograms per milliliter of gp70 by referring to a standard curve obtained from a serum pool of NZB mice.

Histopathology

Kidney samples were collected when mice were moribund or at the end of the experiment at 14 mo of age. Histological sections were stained with periodic acid-Schiff reagent. The extent of glomerulonephritis was graded on a 0 to 4 scale based on the intensity and extent of histopathological changes, as described previously (14). Glomerulonephritis with grade 3 or 4 was considered a significant contributor to clinical disease and/or death.

Statistical analysis

Unpaired comparisons for Tlr7 mRNA expression, B cell proliferative responses, and percentages of MZ B cells and CD69+ monocytes (± SD) among PBMC in 8-mo-old male B6 mice (n = 12) was 8.3 ± 2.2. Mice displaying percentages of monocytes >3 SD above the mean of B6 males (>14.9%) were considered as positive for monocytosis.

Results

Expression of Tlr7 in male B6 mice of the Tlr7+/Yaa, Tlr7−/Yaa, or Tlr7−/y genotype

Similar to previous studies (6, 7), real-time quantitative RT-PCR analysis confirmed a 2-fold increased abundance of Tlr7 mRNA in B cells from male B6 Tlr7+/Yaa mice, as compared with B cells from male B6 Tlr7−/y non-Yaa mice (Fig. 1A). B cells from male B6 Tlr7+/Yaa mice bearing the Tlr7 null mutation on the X chromosome expressed Tlr7 mRNA at levels comparable with those of male B6 Tlr7−/y non-Yaa mice, consistent with having the same copy number of the Tlr7 gene. An enhanced expression of Tlr7 in mice bearing the Tlr7−/−/Yaa genotype was compared with Tlr7−/Yaa and Tlr7−/y B cells (p < 0.05). The specificity of imiquimod as a TLR7 agonist was controlled by the lack of proliferative responses of Tlr7−/− B cells ( ● ). Results (means of three mice ± SD) are expressed as cpm of [3H]Tdr incorporation.
B6 Tlr7+/Yaa mice was reflected by increased proliferative responses of B cells in vitro after stimulation with a TLR7 agonist, imiquimod (Fig. 1B). However, such an augmented response was no longer observed with B cells from male B6 Tlr7+/Yaa mice. Notably, proliferation of B cells in response to a TLR9 agonist, CpG oligonucleotides, was comparable among three different genotypes of male B6 mice (Fig. 1B).

No contribution of Tlr7 gene duplication to loss of MZ B cells in male B6 Tlr7+/Yaa mice

One of the unique cell abnormalities associated with the Yaa mutation is a loss of the CD21highCD23low MZ B cell subset, which is independent of the development of accelerated autoimmune responses (5). To define whether the Tlr7 gene duplication is responsible for this Yaa-linked cell abnormality, we compared the size of the MZ B cell compartment in three different genotypes (Tlr7+/Yaa, Tlr7+/Yaa and Tlr7+/y) of 2-mo-old male B6 mice. Our results, however, showed that the extent of the MZ B cell loss was essentially identical between male B6 Tlr7+/Yaa and Tlr7+/y Yaa mice (Fig. 2). This indicated that the loss of MZ B cells occurring in B6 Tlr7+/Yaa mice was unrelated to the Tlr7 gene duplication, but rather to the duplication of one or several other genes present in the translocated part of the X chromosome.

Contribution of Tlr7 gene duplication to enhanced production of autoantibodies against dsDNA and RNP, but not against chromatin and retroviral gp70 in male B6.Nba2 Tlr7+/Yaa mice

Using the B6.Nba2 model of SLE (13), we determined whether the Tlr7 gene duplication could account for enhanced production of different lupus autoantibodies in mice bearing the Yaa mutation. For this purpose, we established a colony of B6.Nba2 Tlr7+/Yaa mice bearing the Tlr7 null mutation on the X chromosome by intercrossing Tlr7−/− B6 females and B6.Nba2.Yaa males. As shown in Fig. 3A, the Tlr7 mutant gene in B6.Nba2 Tlr7+/Yaa mice is flanked by a ~6-Mb segment from the 129 X chromosome. In addition, analysis of chromosome 1 in B6.Nba2 Tlr7+/Yaa mice confirmed the absence of a distal 129-derived interval in the Nba2

**FIGURE 2.** Comparable reduction of MZ B cells in male 2-mo-old Tlr7+/Yaa and Tlr7−/Yaa B6 mice. Spleen cells from Tlr7+/Yaa, Tlr7−/Yaa and Tlr7+/y B6 mice were stained with a combination of anti-B220, anti-CD21, and anti-CD23 mAb. Representative staining profiles for CD21 and CD23 on B220+ B cells are shown. Percentages of CD21highCD23low MZ B cells (mean of five mice ± SD) are indicated. Differences between Yaa and non-Yaa males were significant (p < 0.01).

**FIGURE 3.** Genetic map of the X chromosome and the telomeric region of the chromosome 1 in B6.Nba2 Tlr7+/Yaa male mice. (A) The segment of ~6 Mb telomeric end of the X chromosome derived from Tlr7−/− 129 mice (gray bar) was introduced into B6.Nba2 Tlr7+/Yaa mice (left). DXMit31 and DXMit32 are located at 160.41 and 163.74 Mb, respectively, from the centromere, and the size of the X chromosome is 166.65 Mb, based on the Ensembl Genome Browser database (www.ensembl.org/Mus_musculus/index.html). The segment of the X chromosome translocated onto the Y chromosome in Yaa mice (■) (6, 7) and all the known genes present in this segment are indicated (right). Gpm6b, glycoprotein m6b; Ofd1, oral-facial-digital syndrome 1 gene homologue; Trappc2, trafficking protein particle complex 2; Rab9, RAB9, member RAS oncogene family; Egfl6, EGF-like-domain multiple 6; Tmsb4x, thymosin β4; Prps2, phosphoribosylpyrophosphate synthetase 2; Frndp4, FERM and PDZ domain containing 4; Ms131, male-specific lethal-3 homolog 1; Arhgap6, Rho GTPase-activating protein 6; Amex, amelogenin X chromosome; Hccs, holocytochrome c synthetase; Mid1, midline 1. B, The NZB chromosomal region (■) present in the B6.Nba2 congenic strain, the Nba2 interval (■, Ref. 36) and the 129 interval (□) implicated in autoantibody production and lupus nephritis (18) are shown. Note the absence of the 129-derived susceptibility interval in B6.Nba2 Tlr7+/Yaa mice. The positions of the microsatellite markers used to establish the genetic map are indicated.
region (Fig. 3B), which has been implicated in susceptibility to anti-nuclear autoantibody production and lupus nephritis (18).

The spontaneous production of IgG anti-nuclear autoantibodies (anti-dsDNA, anti-chromatin, and anti-RNP) and of retroviral gp70 IC in male B6.Nba2 Tlr7−/Yaa, Tlr7+/Yaa, and Tlr7+/Yaa B6.Nba2 mice. Each symbol represents an individual animal (18 Tlr7+/Yaa, 15 Tlr7−/Yaa, and 17 Tlr7+/Yaa B6.Nba2 mice). Results are expressed as units per milliliter for anti-dsDNA, anti-chromatin and retroviral gp70 IC, and as micrograms per milliliter for anti-RNP autoantibodies, and as micrograms per milliliter for gp70 IC. The mean values are indicated by the horizontal line.

FIGURE 4. Serum levels of IgG anti-dsDNA, anti-chromatin and anti-RNP autoantibodies, and gp70 IC in male 5-mo-old Tlr7−/Yaa, Tlr7−/Yaa, and Tlr7+/Yaa B6.Nba2 mice. Each symbol represents an individual animal (18 Tlr7+/Yaa, 15 Tlr7−/Yaa, and 17 Tlr7+/Yaa B6.Nba2 mice). Results are expressed as units per milliliter for anti-dsDNA, anti-chromatin and retroviral gp70 IC, and as micrograms per milliliter for anti-RNP autoantibodies, and as micrograms per milliliter for gp70 IC. The mean values are indicated by the horizontal line.

Because we have previously shown that the percentage of B cells positive for the early activation marker CD69 was higher in 2 mo-old B6.Nba2 mice compared with B6 mice (19), the expression of CD69 on splenic B cells was determined. The percentage of CD69+ B cells in B6.Nba2 Tlr7−/Yaa mice (mean of 5 mice ± SD, 6.6 ± 0.7%) were intermediate when compared with B6.Nba2 Tlr7+/Yaa mice (10.6 ± 1.3%, p < 0.01) and B6.Nba2 Tlr7−/Yaa mice (4.7 ± 0.6%, p < 0.05; Fig. 5A). The number of splenic B cells in B6.Nba2 Tlr7−/Yaa mice (34.3 ± 8.2 × 10^6) appeared to be slightly reduced compared with B6.Nba2 Tlr7+/Yaa mice.

FIGURE 5. Lower incidence of CD69+ B cells and of monocytosis in male B6.Nba2 Tlr7−/Yaa mice compared with male B6.Nba2 Tlr7+/Yaa mice. A, Spleen cells from 2-mo-old B6.Nba2 Tlr7−/Yaa, Tlr7+/Yaa, and Tlr7+/Yaa mice were stained with a combination of anti-B220 and anti-CD69 mAb. Percentages of CD69+ cells in the B220+ population (means of five mice ± SD), with gates based on an irrelevant isotype-matched control conjugate, are shown. Differences between B6.Nba2 Tlr7+/Yaa and Tlr7−/Yaa Yaa mice were significant (p < 0.05). B, PBMC from 8-mo-old B6.Nba2 Tlr7+/Yaa, Tlr7−/Yaa and Tlr7+/Yaa mice were stained with a combination of anti-CD11b and F4/80 mAb. Percentages of CD11b+F4/80+ monocytes in PBMC were determined by flow cytometric analysis. Each symbol represents an individual animal (10–14 mice in each group), and mean values are indicated by horizontal lines. Differences between B6.Nba2 Tlr7+/Yaa and Tlr7−/Yaa males were significant (p < 0.01).
Partial contribution of Tlr7 gene duplication to accelerated development of lupus nephritis in male B6.Nba2 Tlr7+/Yaa mice

Because male B6.Nba2 Tlr7+/Yaa mice developed a lethal form of lupus nephritis with a 50% mortality rate at 14 mo of age (13), we followed the development of lupus nephritis in male B6.Nba2 Tlr7+/Yaa mice and also in male B6.Nba2 Tlr7+/Yaa non-Yaa mice. Although the cumulative rate of mortality due to glomerulonephritis in B6.Nba2 Tlr7+/Yaa male mice was significantly less than that of B6.Nba2 Tlr7+/Yaa male mice (p < 0.05), approximately one-fourth of B6.Nba2 Tlr7+/Yaa males died of lupus nephritis (grade ≥3) by 14 mo of age (Fig. 6). In contrast, none of the male B6.Nba2 Tlr7+/Yaa non-Yaa mice succumbed to lupus nephritis by 14 mo of age, and their glomerular lesions were limited as compared with those observed with male B6.Nba2 Tlr7+/Yaa mice (p < 0.005). These data again indicated that the accelerated development of lupus nephritis in B6.Nba2 Tlr7+/Yaa mice could not be totally explained by the Tlr7 gene duplication alone.

Discussion

The present study was designed to define the contribution of the Tlr7 gene duplication to Yaa-linked accelerated development of SLE, because the Tlr7 gene duplication was proposed as a cause of the Yaa mutation, as a consequence of a translocation from the telomeric end of the X chromosome (which contains the Tlr7 gene) onto the Y chromosome (6, 7). Our analysis of male B6.Nba2 mice of three different genotypes (Tlr7+/Yaa, Tlr7+/Yaa, and Tlr7−/Yaa) demonstrated that the accelerated development of lupus-like autoimmune syndrome in male B6.Nba2 Tlr7+/Yaa mice was partially but not completely prevented by the introduction of the Tlr7 null mutation on the X chromosome. Our data thus indicate that additional gene(s) separate from Tlr7 and present in the translocated X chromosome contribute to the Yaa abnormality.

Our analysis of the development of lupus-like disease demonstrated a significant contribution of the Tlr7 gene duplication to Yaa-linked accelerated development of SLE, as documented by substantial decreases in anti-dsDNA and anti-RNP autoantibody titers and by a lower incidence of lupus nephritis in male B6.Nba2 Tlr7+/Yaa mice. This is consistent with the recent results obtained with FcγRIIB−/− B6 Tlr7+/Yaa mice and TLR7-overexpressing B6 mice (12). However, the contribution of the Tlr7 gene duplication is partial, not complete. Indeed, serum levels of anticromatin autoantibodies and of retroviral gp70 IC in B6.Nba2 Tlr7+/Yaa males were essentially identical with levels in B6.Nba2 Tlr7+/Yaa males and much higher than those in B6.Nba2 Tlr7−/Yaa males. This is consistent with the finding that one-fourth of B6.Nba2 Tlr7+/Yaa male mice still developed a lethal form of glomerulonephritis by 14 mo of age, as anti-chromatin and anti-gp70 autoantibodies have been implicated in the development of murine lupus nephritis (22, 23). It should be stressed that the expression level of Tlr7 in B cells from male Tlr7−/Yaa mice was comparable with that of male Tlr7+/Yaa mice, excluding possible dysregulated expression of the Tlr7 gene translocated onto the Y chromosome. In addition, the absence of the 129-derived chromosome 1 interval implicated in antinuclear autoantibody production and lupus nephritis (18) excludes the possible genetic contribution of this 129-derived susceptibility gene(s) to the development of the residual lupus-like autoimmune syndrome in B6.Nba2 Tlr7+/Yaa mice. Our B6.Nba2 Tlr7+/Yaa mice still carry a ~6-Mb segment from 129 flanking the Tlr7 mutant gene. Because the presence of lupus susceptibility genes on the X chromosome has not yet been analyzed, we cannot completely exclude a putative contribution of 129-derived gene(s) present in this segment to anti-chromatin and anti-RNP autoantibody production.
anti-gp70 autoimmune responses in these mice. However, our preparatory analyses have shown that the production of gp70 IC was completely prevented in female B6.Nba2 Tlr7−/− mice (unpublished observations), thus arguing against this possibility at least for the anti-gp70 response.

Serological analysis of different autoantibodies characteristic of murine SLE in different Tlr7 genotypes of B6.Nba2 mice also defined the differential contribution of TLR7 on autoimmune responses against nuclear Ags. As suggested by the previous studies in Yaa-bearing B6 mice (6, 7), reduced levels of anti-RNP autoantibodies in B6.Nba2 Tlr7−/Yaa mice support the idea that TLR7 is implicated in the autoimmune response against RNA-related autoantigens (8–11). In our studies, serum levels of anti-dsDNA autoantibodies were also diminished in B6.Nba2 Tlr7−/Yaa mice, whereas anti-chromatin autoantibody production was not affected by the TLR7 deficiency. These data suggest that autoimmune responses against chromatin and dsDNA are likely to be controlled by different innate immune receptors. It is also significant that serum levels of gp70 IC were not different between Tlr7−/Yaa and Tlr7−/Yaa B6.Nba2 mice. Because retroviral gp70 is a gene product of endogenous retroviruses, we expected that this autoimmune response would be mediated through TLR7.

The present results cannot rule out the role of TLR7 in the development of auto-antibodies and anti-gp70 autoimmune responses because of the expression of a single copy of the Tlr7 gene in B6.Nba2 Tlr7−/Yaa mice. Importantly, these studies also do not exclude a contribution from the Tlr8 gene, located next to Tlr7, to the autoimmune production and disease, because murine TLR8 was recently shown to be activated by a combination of a TLR7/8 agonist (imidazoquiniline) and oligonucleotides (24), although it is inactive in response to any known human TLR7/8 agonist alone (15, 25, 26). Further analysis in lupus-prone non-Yaa mice deficient in TLR7 or TLR8 would help elucidate the precise role of these innate immune receptors in the development of autoimmune responses and lupus-like disease.

A unique cellular abnormality associated with Yaa-mediated lupus-like autoimmune disease is monocytosis (20). We recently demonstrated that monocytosis is strongly associated with autoantibody production and subsequent development of lupus nephritis and that an NZB-derived susceptibility locus overlapping with Nha2 promoted monocytosis (21). In the current studies, the development of monocytosis was almost completely suppressed in B6.Nba2 Tlr7−/Yaa mice, indicating that the Tlr7 gene duplication is critically involved in the Yaa-associated monocytosis. In addition, other ongoing studies have shown that the development of monocytosis was strongly suppressed in BXSB mice deficient in activating FcγRs, despite a high-level production of autoantibodies (unpublished data). This is consistent with the idea that Yaa-mediated monocytosis may result from an excessive production of monocyte-specific growth factor(s) through macrophages activated by FcγRs in response to IgG IC (27). Thus, it is possible that IgG IC, containing endogenous nuclear Ags and internalized through FcγRs, could excessively activate Yaa-bearing macrophages through subsequent interaction with TLR7, which is expressed at increased levels in endosomes of these mice.

A final point that merits emphasis is that the Tlr7 gene duplication is not responsible for a selective loss of MZ B cells in the spleen occurring in Yaa-bearing mice (5). This is not totally surprising because it has never been reported that TLR7 is implicated in the development and maturation of MZ B cells. Although the precise molecular basis of the loss of MZ B cells in mice bearing the Yaa mutation is still unknown, our preliminary studies revealed that the MZ B cell loss was almost completely abrogated in B6 mice deficient in the Flt3 ligand, in which the development of dendritic cells was markedly compromised (28). In view of a hyperreactive phenotype of Yaa-bearing B cells, as judged by a markedly increased spontaneous IgM secretion associated with an accumulation of plasma cells in the red pulp (5, 29), the loss of MZ B cells in Yaa mice may result from their excessive activation by environmental Ags and apoptotic cells presented by dendritic cells in the MZ (30–32). Because activated MZ B cells can migrate into the red pulp and B cell follicles (30, 33–35), it is possible that the continuous activation of Yaa-expressing MZ B cells may lead to its depletion in the MZ compartment. If so, the Yaa-dependent hyperreactive phenotype of B cells must be determined by gene(s) other than Tlr7 present in the translocated X chromosome, and these gene(s) may contribute to the increased production of autoantibodies. A recent study claimed no significant loss of MZ B cells in male B6 Tlr7−/Yaa mice (12). The difference in results remains unexplained at this time.

In conclusion, our current results suggest that the Yaa-mediated acceleration of SLE includes contributions from the duplication of one or several genes, in addition to Tlr7, present in the translocated part of the X chromosome. An interesting candidate is the Tlr8 gene, which might be implicated in anti-chromatin and anti-gp70 autoimmune responses. In addition, another gene responsible for the possible activation of MZ B cells and hence the hyperreactive phenotype of Yaa-bearing B cells could additionally contribute to the enhancement of overall autoimmune responses. Clearly, further studies to identify the molecular basis of the Yaa mutation will help to determine the target molecules central to the development of SLE, thereby facilitating the design of novel therapeutic strategies in human SLE.

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Disclosures
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