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Dissection of BXSB Lupus Phenotype Using Mice Congenic for Chromosome 1 Demonstrates That Separate Intervals Direct Different Aspects of Disease


To dissect the individual effects of the four non-MHC, autosomal loci (Bxs1 to Bxs4) that contribute to SLE susceptibility in BXSB mice, we generated congenic lines from chromosome 1 on a C57BL/10.Y(BXSB) (B10.Yaa) background for the intervals (values in megabases (Mb)) Bxs1 (46.3-89.2 Mb), Bxs1/4 (20.0-65.9 Mb), Bxs1/2 (64.4-159.0 Mb), and Bxs2/3 (105.4-189.0 Mb). Glomerulonephritis, qualitatively similar to that seen in the parental BXSB strain, developed in three of these congenic strains. Early onset, severe disease was observed in B10.Yaa.BXSB-Bxs2/3 congenic mice and caused 50% mortality by 12 mo. In B10.Yaa.BXSB-Bxs1/4 mice disease progressed more slowly, resulting in 13% mortality at 12 mo. The progression of renal disease in both of these strains was correlated with the level of anti-dsDNA Abs. B10.Yaa.BXSB-Bxs1 mice, despite their genetic similarity to B10.Yaa.BXSB-Bxs1/4 mice, developed a low-grade glomerulonephritis in the absence of anti-dsDNA Abs. Thus, Bxs4 directed an increase in titer and spectrum of autoantibodies, whereas Bxs1 promoted the development of nephritis. The Bxs2 interval was linked to the production of anti-dsDNA Abs without concomitant glomerulonephritis. In contrast, the Bxs3 interval was sufficient to generate classic lupus nephritis in a nonautoimmune–prone strain. Immune phenotype differed between controls and congenics with a significant increase in B220+ cells in BXSB and B10.Yaa.BXSB-Bxs2/3, and an increase in CD4 to CD8 ratio in both BXSB and B10.Yaa.BXSB-Bxs1/4. Disease in the Bxs3 mice was delayed in comparison to the BXSB parental strain, emphasizing the necessity for multiple interactions in the production of the full BXSB phenotype. The Journal of Immunology, 2004, 173: 4277–4285.

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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; ANA, anti-nuclear Ab; gp70C; gp70 immune complex; NZW, New Zealand White; NZB, New Zealand Black.

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the underlying genetic causes of disease. Although some disease intervals are common, others are strain specific. In this respect, the mouse systems seem to reflect the diversity of human disease, and a full understanding of all the murine intervals will aid in the understanding of this complex disorder.

In an attempt to dissect the particularly complicated pattern of disease linkages seen on chromosome 1, we have generated congenic strains of mice for the chromosome 1 loci, Bxs1 to Bxs4. Each of these disease-associated intervals from BXSB has been bred onto the B10 nonautoimmune strain background. The phenotype of these mice has been extensively studied over a period of 12 mo to establish the precise contribution to disease that each interval makes.

Materials and Methods

Mice

Mice were bred and maintained under identical conditions at Imperial College (London, U.K.) from original stocks: BXSB from The Jackson Laboratory (Bar Harbor, ME) and B10 from Harlan Olac (Bicester, U.K.). Both lines have now been maintained for over 20 generations with no change in serological characteristics or mortality. (B10 × BXSB)F1 mice were backcrossed to B10 females and microsatellite markers were used to select male offspring for the next backcross to B10 in a speed congenic approach (18). Lines were fixed by brother-sister mating after six backcrosses. All congenic mice analyzed were male, congenic for the Y chromosome from BXSB and therefore Yaa$, and free from disease pathogens. Age-matched B10 and B10-Yaa$ (B10:Yaa) mice were included as controls for all analyses.

Serological analyses

At least 24 mice for each strain were bled and analyzed at two monthly intervals from 4 mo of age until sacrifice at 12 mo. BXSB mice were tested at 1 mo intervals from 2 to 8 mo of age. ANA levels were measured by indirect immunofluorescence using Hep-2 cells and a fluorescein-conjugated IgG Fc-specific anti-mouse Ab. Samples were screened at a 1/40 dilution and positive samples were titrated to endpoint.

Anti-dsDNA Ab were measured by ELISA using Immunol 2 plates (Thermo Labsystems, Altrincham, U.K.) sensitized with 50 ng of streptavidin (Sigma-Aldrich, Poole, U.K.) and coated with 25 ng of biotinylated gPEM DNA (Promega, Southampton, U.K.) per well. Samples were diluted 1/20 in PBS supplemented with 0.5% BSA and 0.02% NaN3. All samples were bound in duplicate for 3 h at 4°C together with a single nonspecific binding control (no DNA). Anti-dsDNA specific Ab were detected using alkaline phosphatase-conjugated Ab against mouse IgG (Sigma-Aldrich). Results were expressed in comparison to an MRL standard as ELISA units (EU).

Ab to chromatin and ssDNA were measured as previously described (13). The production of autoantibody to gp70 was quantitated as serum levels of gp70-anti-gp70 immune complexes (gp70IC). These complexes were measured by ELISA after precipitation of the serum with polyethylene glycol (average m.w. 6000) as described (19).

Cohorts of mice were compared using Student’s t test or Mann-Whitney U test as appropriate with GraphPad Software (San Diego, CA). Mortality data were assessed using a log rank χ2 test with one degree of freedom.

Autopsy analysis

A cohort of eight mice was sacrificed every 2 mo from 4 mo of age until 10 mo. The mice that had been serially bled were sacrificed at 12 mo. After sacrifice, the spleen was removed and weighed. Kidneys were removed and fixed in formal saline before staining with HE&E for histopathological analysis of glomerulonephritis as previously described (6). Coded kidney sections were scored with respect to the intensity and extent of mesangial matrix increase and glomerular hypercellularity. Samples were graded on a scale 0–4, where 0 signified no histological changes, 1 indicated <25% abnormal glomeruli, 2 corresponded to 25–50%, grade 3 was 51–75%, and 4 indicated >75%. Grades 2–4 were considered positive for glomerulonephritis, grade 0 was negative. Snap-frozen kidneys were cryosectioned and stained with goat anti-mouse IgG-FITC (Sigma-Aldrich), and goat anti-mouse C3-FITC (ICN Pharmaceuticals, Basingstoke, U.K.). Sections were also stained with a polyclonal anti-goat/sheep IgG-FITC (Sigma-Aldrich) to detect nonspecific binding. All sections were washed a minimum of four times in PBS to ensure that background staining was minimized.

Flow cytometric analysis

The following directly conjugated mAbs were used during flow cytometric analysis: anti-CD4-allophycocyanin, anti-CD8 TriColor-conjugated (TC), anti-CD45RB-PE, and anti-B220-FITC. Isotype-matched controls rat IgG2a-Tc, rat IgG2a-allophycocyanin, rat IgG2a-FITC, and rat IgG2a-PE (all from Caltag Laboratories, Burlingame, CA), anti-B220-FITC (RA3-6B2), and anti-CD11b-PE (BD Biosciences, San Diego, CA).

To examine the expression of surface Ags, cells (2 × 10⁷) were incubated with the appropriate directly conjugated Ab for 30 min on ice. Cells were washed twice with ice-cold FACS buffer (PBS, 1% FCS, 0.05% NaN₃) and fixed with FACS lysis solution (Sigma-Aldrich). Cells were subsequently analyzed on a FACScan flow cytometer (BD Biosciences) using CellQuest software. Dead cells and debris were excluded by forward and side scatter gating.

Results

Generation of mouse strains congenic for the BXSB lupus-susceptibility loci, Bxs1, 2, 3, and 4

Four congenic lines have been generated using a speed congenic approach (18). A panel of 117 microsatellite markers was used to select breeding pairs (full list available on request to authors). The mice generated (Fig. 1) were named after the BXSB lupus-susceptibility intervals they contain (6, 7). B10.Yaa$BXSB-Bxs1/4 (B10.Yaa.Bxs1/4), B10.Yaa$BXSB-Bxs2/3 (B10.Yaa.Bxs2/3), B10.Yaa$BXSB-Bxs2/3 (B10.Yaa.Bxs3/3), and B10.Yaa$BXSB-Bxs3/4 (B10:Yaa.Bxs3/4) were defined by the inheritance of BXSB-derived microsatellite markers from D1Mit5 (20.02 Mb) to D1Mit1000 (65.95 Mb). B10.Yaa.Bxs1/4 was bred by selecting BXSB-derived microsatellite markers from D1Mit235 (46.3 Mb) to D1Mit305 (89.2 Mb). B10.Yaa.Bxs2/3 covered the BXSB-derived microsatellite markers from D1Mit303 (64.5 Mb) to D1Mit33 (159.0 Mb). B10.Yaa.Bxs3/4 contained the BXSB-derived interval from D1Mit135 (105.6 Mb) to D1Mit223 (189.0 Mb). All congenic lines were Yaa$. Control B10.Yaa mice were generated by 10 generations of backcrossing to B10 females and confirmation of the absence of BXSB autosomes using our panel of 117 microsatellite markers.

Accelerated mortality was observed with the Bxs3 interval

Significant mortality (log rank test) was observed for B10.Yaa.Bxs2/3 mice (Fig. 2), which had 52.1% mortality (25/48) by 12 mo (p < 0.0001 from all congenic and control strains). None of the other congenic strains showed a difference in mortality from the B10.Yaa congenic strain (2/48). However, B10.Yaa.Bxs1/4 (6/47) and B10.Yaa.Bxs1/2 (4/47) showed significantly increased mortality with respect to B10 (0/48) control mice (p = 0.008 and p = 0.033, respectively).

Bxs3 and Bxs4 intervals directed the development of a broad spectrum of autoantibodies

All strains of mice congenic for the BXSB Y chromosome developed elevated titers of anti-chromatin Abs compared with those seen in B10 control mice (Fig. 3A). Onset of autoantibody production was markedly earlier in B10.Yaa.Bxs1/2, although the end-point titer was similar. In contrast, levels of anti-chromatin Abs in the B10.Yaa.Bxs2/3 were significantly higher at all time points than for any other strain studied and it is likely that these Abs are detectable before the initial 4 mo time point at which samples were taken. A similar pattern emerged for ANA. Production was greatest in B10.Yaa.Bxs2/3 mice, and this trait was evident from 4 mo onwards (Fig. 3B). B10.Yaa.Bxs1/4 developed significantly higher titers of ANA than B10.Yaa.Bxs1/2, B10.Yaa.Bxs1/2, or B10.Yaa mice at 10 mo, whereas the levels of ANA in these three strains were indistinguishable. ANA titers in B10.Yaa.Bxs1/4 mice were
FIGURE 1. Map of chromosome 1 showing microsatellite markers used for breeding B10. Yaa.Bxs1, B10. Yaa.Bxs1/4, B10. Yaa.Bxs1/2, and B10. Yaa.Bxs2/3 congenic strains of mice. The scale indicates positions in megabases according to Ensembl mouse genome assembly National Center for Biotechnology Information 32 (http://www.ensembl.org/Mus_musculus/). The BXSBS interval (■) selected for inclusion in the congenic strain and the interval (□) between the selection markers for which inclusion or exclusion is not proven are indicated. Locations of previously identified BXSB disease-associated loci are indicated (6, 7).

FIGURE 2. Kaplan-Meyer survival plot for congenic and control mice. The 47 or 48 mice were followed for each strain to 52 wk. The significance of the difference between the congenic strain and B10. Yaa control are indicated; ***, p ≤ 0.001. B10 mice (●), B10. Yaa mice (○), B10. Yaa.Bxs1/4 (▲), B10. Yaa.Bxs1 (△), B10. Yaa.Bxs1/2 (□), B10. Yaa.Bxs2/3 (■) are depicted.

B10. Yaa.Bxs1 were indistinguishable from B10 or B10. Yaa controls. A similar pattern was observed for the production of anti-DNA Abs, which were elevated from 4 mo in B10. Yaa.Bxs2/3 and by 12 mo for B10. Yaa.Bxs1/4 mice (Fig. 3). However, B10. Yaa.Bxs1/2 congenic mice also developed low levels of anti-DNA Abs at 12 mo, which were significantly higher than those seen in B10 or B10. Yaa. For both anti-DNA Abs, the levels observed in B10. Yaa.Bxs2/3 mice were considerably higher than in any other strain.

No significant levels of gp70 or gp70ICs were detected in sera from any of the congenic strains at any time point. Maximum gp70 titers for any individual from each line were 0.36 μg/ml for B10. Yaa.Bxs1/4 (mean = 0.27 μg/ml), 0.41 μg/ml (mean = 0.23 μg/ml) in B10. Yaa.Bxs1, 0.57 μg/ml (mean = 0.24 μg/ml) for B10. Yaa.Bxs1/2, and 0.45 μg/ml for B10. Yaa.Bxs2/3 (mean = 0.34 μg/ml), compared with 27.6 μg/ml (mean = 14.4 μg/ml) for 4-mo-old BXSB mice. A level of gp70IC of 0.53 μg/ml was observed in a single B10. Yaa.Bxs1/2 congenic mouse at 10 mo (BSXBS 4 mo mean = 1.34 μg/ml), but for the remainder, levels were undetectable. None of the levels of gp70 or gp70IC in the congenic lines were significantly different from those observed in B10 mice (mean gp70 = 0.40 μg/ml and gp70IC = 0.02 μg/ml).

Bxs1 and Bxs3 were independently associated with the development of nephritis

Histological studies of B10. Yaa.Bxs2/3, B10. Yaa.Bxs1/4, and B10. Yaa.Bxs1 mice showed an age-dependent glomerulonephritis that was qualitatively similar to the lesions observed in BXSB mice (Fig. 4). These mice developed a focal mesangial hypercellularity accompanied by matrix increase. B10. Yaa.Bxs2/3 mice developed a focal global mesangial hypercellularity accompanied by matrix increase from 4 mo of age onward. By 12 mo of age, the glomerulonephritis was severe (grade 4) in 16 of 19 mice examined (Fig. 3E). Onset of glomerulonephritis was much slower in B10. Yaa.Bxs1/4 mice; a moderate level of glomerulonephritis was detectable at 6 mo (median grade = 2) rising to a median grade 3...
FIGURE 3. Phenotypic analysis of congenic mice. Mice were analyzed at 4, 6, 8, 10, and 12 mo for serum anti-chromatin Abs (A), ANA (B), anti-ssDNA Abs (C), anti-dsDNA Abs (D), glomerulonephritis (E), and total spleen weight (F). The mean and SEM values for 32 mice are plotted for serological data (A–D) and split scales have been used (A–C). Values from at least eight mice at each time point are plotted (E and F). B10 mice (●), B10.Yaa mice (○), B10.Yaa.Bxs1/4 (▲), B10.Yaa.Bxs1/2 (■), and B10.Yaa.Bxs2/3 (■) are depicted. The significance of the difference between the congenic strain and B10.Yaa control are depicted as *, p ≤ 0.05, **, p ≤ 0.01, ***, p ≤ 0.001. Mouse strains were compared using Student’s t test (spleen weight, anti-chromatin Abs, glomerulonephritis) or Mann-Whitney U test (ANA, anti-ssDNA, anti-dsDNA).
Immunostaining identified significant deposition of IgG in the glomeruli of B10.Yaa.Bxs2/3 and B10.Yaa.Bxs1/4 lines as well as in control BXSB mice, with minor deposition in B10.Yaa.Bxs1, mirroring the nephritis grades. For B10.Yaa.Bxs2/3 mice from 8 mo of age onward, staining intensity was comparable to that seen in BXSB control mice. Activated complement, as indicated by C3 deposition, was strikingly visible in the glomeruli of the BXSB mice, and to a lesser extent, in B10.Yaa.Bxs2/3 mice. Other than this measure of severity, there were no qualitative differences between the nephritis identified in any of the congenic strains and BXSB.

**Splenomegaly was associated with Bxs3 and Bxs4**

Splenomegaly was detectable from 4 mo in B10.Yaa.Bxs2/3, B10.Yaa.Bxs1/4, and B10.Yaa mice from 8 mo but not until 10 mo for B10.Yaa.Bxs1 and B10.Yaa.Bxs1/2 (Fig. 3F). However, increased spleen weight was only significantly greater than that observed for the B10.Yaa controls in the B10.Yaa.Bxs2/3 and B10.Yaa.Bxs1/4 strains. Median spleen weight was higher at all time points for B10.Yaa.Bxs2/3 in comparison to all other strains, whereas B10.Yaa.Bxs1/4 was significantly higher than B10.Yaa control mice only at the 12 mo time point.

**Altered immune phenotype reflected disease severity in congenic mice**

Flow cytometric analysis and total splenocyte counts revealed differences between the lymphocyte populations in the congenics and the control mice (Table I). The absolute number of lymphocytes in BXSB and B10.Yaa.Bxs2/3 far exceeded those in all the other strains by as much as 2- and 3-fold, respectively. BXSB and B10.Yaa.Bxs2/3 demonstrated a significant increase in the number of B220⁺ lymphocytes (p = 0.0007 and p < 0.0001, respectively, as determined by flow cytometry), whereas the CD4 to CD8 ratio was significantly increased in both BXSB and B10.Yaa.Bxs1/4. Analysis of the percentage of total lymphocytes in BXSB, B10.Yaa.Bxs2/3, B10.Yaa.Bxs1/4, and B10.Yaa clearly demonstrated a substantial increase in the percentage of B220⁺ cells at later time points in both congenics and the parental BXSB strain compared with B10.Yaa (Fig. 5). Furthermore, as time progressed, the increase in B220⁺ cells was first accompanied by a decrease in CD8⁺ cells, followed by a decline in CD4⁺ cells. The increased percentage of B220⁺ cells in both BXSB and B10.Yaa.Bxs2/3 was mirrored by an increase in absolute cell number, clearly reflecting cell division and expansion. In contrast, although there is a low percentage of CD3⁺ splenocytes in these mice, the absolute cell number still exceeded that of the control mice. The data for the other congenics did not differ significantly from the B10.Yaa control and the data is not shown.

![Image](https://example.com/image.png)

**Table I. Flow cytometric analysis of splenocytes**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>CD3⁺</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>B220⁺</th>
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<tbody>
<tr>
<td>B10</td>
<td>35.7 ± 4.0</td>
<td>19.8 ± 2.6</td>
<td>15.8 ± 3.2</td>
<td>19.7 ± 2.2</td>
</tr>
<tr>
<td>BXSB</td>
<td>146.0 ± 20.8**</td>
<td>110.9 ± 15.7***</td>
<td>33.9 ± 3.2**</td>
<td>117.2 ± 19.7*</td>
</tr>
<tr>
<td>B10.Yaa</td>
<td>36.2 ± 3.7</td>
<td>19.8 ± 2.0</td>
<td>16.5 ± 1.4</td>
<td>36.8 ± 3.7</td>
</tr>
<tr>
<td>B10.Yaa.Bxs1/4</td>
<td>29.9 ± 2.8*</td>
<td>19.7 ± 1.8</td>
<td>9.7 ± 1.2*</td>
<td>39.4 ± 3.2</td>
</tr>
<tr>
<td>B10.Yaa.Bxs1</td>
<td>51.4 ± 0.2</td>
<td>22.8 ± 2.0</td>
<td>26.6 ± 1.8*</td>
<td>17.0 ± 1.1**</td>
</tr>
<tr>
<td>B10.Yaa.Bxs1/2</td>
<td>41.0 ± 3.2</td>
<td>17.8 ± 2.8</td>
<td>22.8 ± 1.7*</td>
<td>35.8 ± 1.7</td>
</tr>
<tr>
<td>B10.Yaa.Bxs2/3</td>
<td>23.9 ± 2.5</td>
<td>11.3 ± 1.6*</td>
<td>14.2 ± 1.0</td>
<td>111.8 ± 6.5***</td>
</tr>
</tbody>
</table>

* Results represent between three and six mice 10–12 mo of age with the exception of BXSB, which were 6 mo old. Results are expressed as mean ± SEM of total splenocytes (×10⁸). The significance of the difference (calculated by t tests) between BXSB or the congenic strain and B10.Yaa control is indicated by *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001.
The activation status of CD4+ lymphocytes in the peripheral blood of the congenic and control mice at 6 mo was determined by two-color flow cytometry (data not shown). The percentage of activated CD4+ cells (CD4+CD45RBlow) was elevated in BXSB (67.03%), B10.Yaa.Bxs2/3 (69.53%), and B10.Yaa.Bxs1/4 (59.59%) compared with B10.Yaa (41.28%). No difference was seen with the other congenics.

There was a slight increase of the proportion of B-1b cells in the spleen by 12 mo of age in all mice carrying Yaa (Table II). The B-2 cell population remained constant for all mice with the exception of the B10.Yaa.Bxs2/3 mice in which only one-third of the splenocytes were B220+. Approximately 25% of the cells in this strain did not stain for B220, CD11b, CD4, nor CD8 (compared with around 10% in all other strains) and were therefore likely to be plasmocytes, myeloid precursors and/or the newly described Ag capturing cells (20). Further characterization of this nonstaining cell population is ongoing. The monocyte/macrophage population also tended to be elevated in all Yaa+ mice; however, this was significant only in BXSB mice (starting from 5 mo of age) and B10.Yaa control is indicated by * (p ≤ 0.05, **, p ≤ 0.01, *** p ≤ 0.001).

**Discussion**

In this study we have shown that the production of specific subsets of autoantibodies can be caused by the effect of Yaa on a nonautoimmune-prone strain of mice. These results would appear to contradict previous reports that Yaa had no measurable effect outside of autoimmune-prone mice (4). However, the two Abs in question were not studied in the C57BL/6.YBXC congenic strain, and it is likely that these mice also develop anti-chromatin Ab and ANA. B10.Yaa mice do not progress to develop anti-DNA Ab or glomerulonephritis. However, the other congenic strains presented in this study are consomic for the BXSB Y chromosome and so will have a partial autoimmune phenotype driven by Yaa that develops irrespective of the presence of additional chromosome 1 disease-susceptibility intervals (summarized in Table III). In essence, the congenic strains presented are bicongenic, possessing both a specific chromosome 1 interval and Yaa.

B10.Yaa.Bxs1/2 mice did not develop any significant renal lesions, but by 12 mo had significantly elevated levels of anti-dsDNA Abs, similar to those observed for B10.Yaa.Bxs1/4 mice. However, we have followed these mice to 18 mo of age and can detect no significant glomerulonephritis in this strain. Thus it would appear that simply adding a significant anti-dsDNA Ab titer to the Yaa effect (anti-chromatin Ab and ANA) is insufficient to produce glomerulonephritis.

In contrast, B10.Yaa.Bxs1 mice developed significant glomerulonephritis, which was still increasing in severity at 12 mo. Aside from anti-chromatin Ab and ANA, which were also expressed in B10.Yaa, this strain did not develop any significant levels of any of the autoantibodies reported in this study. From these data, it is not possible to determine whether Bxs1 is capable of functioning in the absence of an epistatic interaction with Yaa or Bxs4. However, it is certain that a gene (or genes) in this interval is capable of functioning in the absence of any known lupus autoantibodies. This is consistent
with a hypothesis that there are genes, such as Brx1, that cause nephritis by an alternative route rather than anti-DNA Ab titer. Whether this is due to the production of novel Abs, as appears to be the case in the NZM2328 model (21, 22) or whether the locus at Bxs1 can cause nephritis in the absence of autoantibodies remains to be determined. However, in a backcross of (NZW × BXSB)F1 mice to NZW, neither anti-cardiolipin nor anti-platelet Ab showed linkage to this distal region of chromosome 1 (23). The specificity of any Ab production associated with this interval would therefore be very rare within the lupus phenotype. We are generating B10.Yaa.Bxs1 to address this question.

There is considerable genetic overlap between the B10.Yaa.Bxs1/2 and B10.Yaa.Bxs1 mice, which allow us to closely define the genetic intervals encompassing Bxs1. It must be located in the region that is unique to the B10.Yaa.Bxs1 strain in comparison to the B10.Yaa.Bxs1/2 mice (which develop no glomerulonephritis), specifically the region between D1Mit325 and D1Mit303, an interval of some 18 Mb. This confirms our earlier linkage data for the Bxs1 locus (6), and that this interval is probably not present in the B10.Yaa.Bxs1/2 strain. An interval with a linkage to splenomegaly has been identified, with a peak at D1Mit46 (77 Mb) in a (BXSB × NZW)F2 intercross (24). Our study suggests that this locus is distinct from Bxs1 because there was no linkage to nephritis in the intercross and the linkage peak is 15 Mb telomeric to Bxs1. It is possible that Bxs2 is the same locus because the B10.Yaa.Bxs1/2 mice have a mild splenomegaly compared with B10. However, the fact that all the congenic mice described in this study showed this level of splenomegaly and there was an absence of linkage to anti-dsDNA Ab production, a characteristic of the Bxs2 interval, in the (BXSB × NZW)F2 intercross does mitigate against this. One explanation may be that interactions with different background genes in the two studies, B10.Yaa in this study and BXSB/NZW in the intercross cause different phenotypic expression. Alternatively, the interval described in the (BXSB × NZW)F2 intercross may be a novel and separate linkage.

The B10.Yaa.Bxs1 mice do not develop high levels of anti-dsDNA Ab, therefore, the causative basis of this phenotype must lie in the interval exclusive to B10.Yaa.Bxs1/2 mice, namely between D1Mit305 and D1Mit33. This 54 Mb interval is precisely the one that we had previously identified as Bxs2, and found to be associated with several aspects of the BXSB phenotype. This interval may also contribute to the very early onset of anti-chemokatin Ab production in B10.Yaa.Bxs1/2 mice, a phenotype also observed in B10.Yaa.Bxs2/3 mice, indicating that this trait may lie in the overlap between these strains, D1Mit135 to D1Mit33.

The Bxs1 nephritis locus is, however, present in B10.Yaa.Bxs1/4 congenic mice, and therefore is likely be the etiological agent for the significant glomerulonephritis observed in this strain. However, in addition, B10.Yaa.Bxs1/4 mice also developed significant levels of anti-dsDNA and anti-ssDNA Ab. There is very limited genetic overlap between B10.Yaa.Bxs1/4 and B10.Yaa.Bxs1/2, encompassed by the Bxs1 strain, which does not develop anti-dsDNA Ab. Therefore, there must be a different basis for the anti-dsDNA Abs observed in these strains. Furthermore, Bxs1 and Bxs2 did not lead to the production of anti-ssDNA Ab, so this must be a function of the unique region in B10.Yaa.Bxs1/4, although it may be dependent on the presence of the Bxs1 interval. This anti-DNA Ab locus is therefore restricted to the interval between D1Mit3 and D1Mit235 (26 Mb), and in the interval we previously designated Bxs4. Kidney disease in B10.Yaa.Bxs1/4 mice was of a significantly higher grade than that observed for B10.Yaa.Bxs1 mice. This is most likely a reflection of the higher levels of autoantibodies in this congenic line, driving the development of disease. We have previously shown a chromosome 13 locus, Bxs6, was capable of driving nephritis via the production of gp70 and gp70IC (8) and a similar mechanism of immune complex load is probably at play here.

The high levels of autoantibodies and glomerulonephritis found in B10.Yaa.Bxs2/3 mice confirm that the telomeric region of chromosome 1 in BXSB behaves as a major SLE locus. In this respect, Bxs3 presents a similar phenotype to the extensively studied Sle1 (9). Indeed, Sle1 in a bicongenic strain with Yaa has a similar, very severe phenotype, to that generated by the B10.Yaa.Bxs2/3 line (25). It has been demonstrated for Sle1 that multiple genetic effects map to this region (26), and we suspect that Bxs3 will be similarly complex. Both our own studies on BXSB (6, 7) and work on the NZB/NZW model, have implicated Bxs3/Nba2/Sle1 in the production of high levels of all Abs (10, 25). This lends weight to the argument that this interval is driving a similar process in the different strains. However, a certain amount of caution must be maintained in comparing these data because it is known that, for example, the Fcgr2 locus in Sle1 is derived from NZW (27), which does not show the same decrease in expression of the receptor protein as has been shown in BXSB (Bxs3) or indeed NZB (Nba2) (28).

One unique feature of the BXSB mice compared with other lupus-prone mouse strains is a monocytosis (29). There was a suggestion from the flow cytometric analysis of splenocytes that this may be linked specifically to the Bxs2/3 interval, with an increase in the proportion of monocytes in the B cell fraction from 3% to 5% in all the strains to 12.5% in the B10.Yaa.Bxs2/3 line. Only the parental BXSB strain showed a similar increase. Thus, although the Bxs3/Sle1/Nba2 interval generates a similar phenotype in all lupus strains, it is likely that the BXSB interval has an additional role in the disease process.

The disease in B10.Yaa.Bxs2/3 mice matched that in the parental BXSB in every respect apart from the time course. In the parental BXSB mice, 50% mortality occurred at around 6 mo (1), whereas in B10.Yaa.Bxs2/3 mice this did not occur until 12 mo, even though by then the levels of almost all autoantibodies were

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>BXS Y Chromosome</th>
<th>BXS Chromosome 1 Interval and Borders (Mb)</th>
<th>Anti-Chromatin Ab</th>
<th>Anti-ssDNA Ab</th>
<th>Anti-dsDNA Ab</th>
<th>Nephritis</th>
<th>Splenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10.Yaa</td>
<td>+</td>
<td>(0) 20.0 – 65.9 (71.0)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B10.Yaa.Bxs1/4</td>
<td>+</td>
<td>(40.5) 46.3 – 89.2 (105.6)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B10.Yaa.Bxs1/2</td>
<td>+</td>
<td>(53.0) 64.4 – 159.0 (163.0)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B10.Yaa.Bxs2/3</td>
<td>+</td>
<td>(89.2) 105.4 – 189.0 (~195.9)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BXSB (6 mo)</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The phenotype data shown refers to mice at 12 mo of age, with the exception of BXSB, which were measured at 6 mo. −, Negative for this genetic interval or phenotypic trait; +, mild; ++, moderate; +++, severe phenotype.
higher than those observed in the parental strain. B10.Yaa.Bxs2/3 mice do not contain the nephritis gene, Bxs1. The slower progression of disease in the B10.Yaa.Bxs2/3 congenics suggests that additional factors are necessary to recapitulate the full BXSX phenotype. The data presented support Bxs1 as one of these factors. Furthermore, these mice do not contain Bxs6, and gp70IC certainly play a role in the development of severe glomerulonephritis (8).

A number of proteins have been implicated in the pathogenesis of murine SLE from targeted gene deletion, whereas human association studies have lent support to these data. Of these, some instances loss of expression ameliorated autoimmune disease, e.g., CD28 (30), or had no measurable phenotype, e.g., Ifi202 (31). Others, such as DNaseI (32), Lyn (33), and CD22 (34) are not encoded on chromosome 1 and therefore could not be the causative susceptibility genes in BXSX. However, a number of strong candidates remain including CItla-4 ((35, 36), Pdcd-1 (37, 38), and the FcγRs (39, 40). In addition, expression profiling data have implicated Ifi202 in the disease process (41). By comparing the phenotypes of the knockout mice with the phenotypes of the congenic lines containing these genes their potential as susceptibility loci in BXSX can be determined. Thus, targeted deletion of CItla-4 (62.5 Mb), which lies in the Bxs1 interval, resulted in a massive CD4+ expansion and rapid death within 4 wk (35, 42). There is a slight increase in CD4+ T cell number in the B10.Yaa.Bxs1 mice (Table I), but not in B10.Yaa.Bxs1/4 mice. Thus, the data do not rule out a role for CItla-4, but there is no strong support for a significant decrease in CItla-4 function/expression in these mice. Indeed, preliminary sequence analysis has not shown any changes between BXSX and B10 (data not shown).

For Pdcd-1 (encoded at 95.9 Mb), the data are more definitive. Pdcd-1 knockout mice developed lupus-like proliferative arthritis and glomerulonephritis, but did not mount an Ab response to dsDNA (37). This gene lies in the Bxs2 interval, which was associated with the development of low-level anti-dsDNA Abs. In addition, BXSX do not develop arthritis. Therefore it seems unlikely that Pdcd-1 plays a direct role in disease susceptibility in this model.

Targeted deletion of Fcgr2 resulted in increased total numbers of T cell and B cell with a concomitant increase in T cell and B cell activation (39). This situation was mirrored in the B10.Yaa.Bxs2/3 line. There was an increase in activation markers on CD4+ cells (CD69) with the knockout mice, although there was no increase in the CD4+ to CD8+ T cells ratio as reported for the Fcgr2 knockout model. We have previously demonstrated that the Fcgr2 promoter has two regions deleted in BXSX compared with B10 (28) and this directly affects expression levels of the protein in macrophages and activated B cells, hence reducing function (28, 43). It is probable that the Fcgr2 gene plays a significant role in the disease susceptibility of the Bxs3 interval. Ifi202 has been implicated in the NZB mouse by microarray analysis and we have preliminary data to support differential expression in the BXSX strain (M. E. K. Haywood, S. J. Rose, M. J. Lees, G. Fu, M. J. Walport, and B. J. Morley, manuscript in preparation). Thus, it is likely that both FcγRII and Ifi202 play important roles in the susceptibility of the Bxs3 interval.

Flow cytometric analysis of splenocytes and peripheral blood cells in the congenics and control mice have revealed striking differences in both percentage lymphocyte populations and absolute cell numbers. A clear correlation between increasing B220+ lymphocytes and disease progression is seen in both BXSX and B10.Yaa.Bxs2/3, with a comparable decrease in percentage (although not absolute cell number) of CD3+ lymphocytes. Although the percentage of CD8+, and subsequently CD4+, cells decreased, the CD4+ cells present were shown to be highly activated as revealed by an increase in CDRB+ expressing cells compared with B10.Yaa control mice. Thus, while there is an increase in absolute cell number, this may suggest either a slower rate of CD3+ lymphocyte expansion compared with B220+ cells, or alternatively a higher rate of activation induced cell death in the CD3+ population. These data strongly suggest a role for both B220+ and CD4+ cells in disease progression, however, the precise role of each has yet to be defined. Work is currently underway to further characterize the involvement of both CD3+ and B220+ lymphocyte populations and furthermore to refine the role of CD4+ subsets in disease onset and progression.

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References


