Anti-Sm B Cell Differentiation in Ig Transgenic MRL/Mp-\textit{lpr/lpr} Mice: Altered Differentiation and an Accelerated Response

Sandra Santulli-Marotto, Ye Qian, Stacy Ferguson and Stephen H. Clarke

\textit{J Immunol} 2001; 166:5292-5299; doi: 10.4049/jimmunol.166.8.5292

http://www.jimmunol.org/content/166/8/5292

References
This article cites 54 articles, 36 of which you can access for free at: http://www.jimmunol.org/content/166/8/5292.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Anti-Sm B Cell Differentiation in Ig Transgenic MRL/Mp-lpr/lpr Mice: Altered Differentiation and an Accelerated Response

Sandra Santulli-Marotto,2 Ye Qian, Stacy Ferguson,3 and Stephen H. Clarke4

To determine the regulation of B cells specific for the ribonucleoprotein Sm, a target of the immune system in human and mouse lupus, we have generated mice carrying an anti-Sm H chain transgene (2-12H). Anti-Sm B cells in nonautoimmune 2-12H-transgenic (Tg) mice are functional, but, in the absence of immunization, circulating anti-Sm Ab levels are not different from those of non-Tg mice. In this report, we compare the regulation of anti-Sm B cells in nonautoimmune and autoimmune MRL/Mp-lpr/lpr (MRL/lpr) and bcl-2-22-Tg mice. Activation markers are elevated on splenic and peritoneal anti-Sm B cells of both nonautoimmune and autoimmune genetic backgrounds indicating Ag encounter. Although tolerance to Sm is maintained in 2-12H/bcl-2-22-Tg mice, it is lost in 2-12H-Tg MRL/lpr mice, as the transgene accelerates and increases the prevalence of the anti-Sm response. The 2-12H-Tg MRL/lpr mice have transitional anti-Sm B cells in the spleen similar to nonautoimmune mice. However, in contrast to nonautoimmune mice, there are few if any peritoneal anti-Sm B-1 cells. These data suggest that a defect in B-1 differentiation may be a factor in the loss of tolerance to Sm and provide insight into the low prevalence of the anti-Sm response in lupus. The Journal of Immunology, 2001, 166: 5292–5299.

Systemic lupus erythematosus (SLE)3 is characterized by the production of autoantibodies specific for a variety of self-Ags, many of which are nuclear in origin, including DNA, histones, and ribonucleoproteins (RNPs) (1). The factors that lead to a breakdown in B cell tolerance to these Ags remain elusive. Recent progress in identifying the normal mechanisms of B cell tolerance has come from the use of mice transgenic (Tg) for Ig genes that encode anti-self Abs. These models have focused on B cells specific for neo-self-Ags and self-Ags not targeted in lupus (hen egg lysozyme and MHC class I) (2, 3), as well as lupus-specific Ags, most prominently, DNA and Ig (4, 5). They have revealed a variety of B cell tolerance mechanisms in nonautoimmune mice that includes central deletion, peripheral deletion, anergy, receptor editing, and ignorance (2–4, 6–9).

Autoantibodies reactive to nuclear components are characteristic of several autoimmune syndromes, such as Sjögrens syndrome and rheumatoid arthritis, in addition to SLE (1). For example, the anti-dsDNA response occurs in more than one autoimmune syndrome (1), whereas the anti-Sm response is unique to SLE (1). Understanding why tolerance to nuclear Ags is lost in these diseases is complicated by the fact that not all individuals with a given disease develop the same autoantibodies. For example, the prevalence of anti-Sm and anti-dsDNA in human SLE is ~25 and 75%, respectively (1). This disparity holds in mouse models of lupus. The disease in MRL/Mp-lpr/lpr (MRL/lpr) mice closely mimics human lupus (10–12). It begins in adults, and the prevalence of the responses to Sm and dsDNA are ~25 and >90%, respectively (1, 13). Genetic, maternal, and environmental factors have been ruled out in controlling the prevalence of the anti-Sm response (11). Our studies on gene use by anti-Sm B cells of MRL/lpr mice indicate that a limitation in V H or V k gene use or in the acquisition of particular somatic mutations are not factors in the low prevalence (14).

Mice of only one other strain, bcl-2-22-Tg mice, are known to develop circulating anti-Sm Abs (15). B cells in these mice overexpress bcl-2 at all stages of development, significantly increasing B cell longevity and number and prolonging responses to foreign Ags (15). Bcl-2-22 mice produce several different autoantibodies in addition to anti-Sm and develop glomerulonephritis. The effects of bcl-2 on central and peripheral tolerance have been studied and, in general, central tolerance remains intact, but peripheral tolerance can be disrupted in some situations (16–18).

To follow anti-Sm B cell regulation, we have generated anti-Sm Ig H chain-Tg mice (2-12H Tg) (19). The transgene encodes an unmutated H chain derived from hybridoma 2-12 of MRL/lpr origin. About 30% of splenic B cells of 2-12H Tg mice are anti-Sm. Most are transitional B cells, but some may be mature B-2 cells. Most non-Sm-binding B cells are B-2. The preponderance of transitional over mature anti-Sm B cells suggests a deficiency of anti-Sm B cells to either differentiate to or persist as B-2 cells. Splenic anti-Sm B cells can differentiate to B-1, and, in the peritoneum, anti-Sm B cells account for ~30% of the B-1 repertoire (20). Although serum anti-Sm levels in Tg mice are equivalent to those of non-Tg littermates, some anti-Sm B cells are functional, as immunization with Sm-containing Ags induces an Ab response (19). Thus, anti-Sm B cells appear to be ignorant, and we have proposed that their differentiation to B-1 helps maintain ignorance (20).

To investigate the loss of tolerance in autoimmune mice, we have generated 2-12H-Tg MRL/lpr mice and 2-12H/bcl-2 double-Tg mice and compared their anti-Sm B cells with those of...
nonautoimmune Tg mice. The anti-Sm B cells of both autoimmune and nonautoimmune Tg mice have elevated levels of activation markers, indicating that they have encountered Ag. Although the bcl-2 transgene appears to have no affect on anti-Sm regulation, the anti-Sm response is accelerated and its prevalence increased to 100% in 2-12H-Tg MRL/lpr mice. Phenotypic analysis indicates that differentiation proceeds normally to the transitional cell stage in MRL/lpr mice and that anti-Sm B-1 cells are absent.

Materials and Methods

Mice

The generation and screening of the 2-12H-Tg mice has been described previously (19). The 2-12H-Tg MRL/lpr mice were generated by breeding 2-12H-Tg × MRL/lpr-Ighb 3 and/or intact anti-IgM a or anti-IgM b (BD PharMingen, San Diego, CA). Assays were developed with 1 mg/ml with alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Bio-Technology Associates). Detection of anti-Sm by ELISA was done as previously described (14). Briefly, 96-well polyvinyl chloride plates were coated with Sm (Immuno-Biological Laboratories, Gardena, CA), followed by streptavidin-alkaline phosphatase (Southern Bio-Technology Associates). Mouse sera were serially diluted in BBS/BSA/Tween 20 and then added to Sm-coated plates in duplicate. IgM was quantitated by adding sera to plates blocked with BBS/BSA/Tween 20. All washes were done with BBS.

Results

Anti-Sm B cells predominate in young mice

To determine the effect of the autoimmune background of MRL/lpr mice on the differentiation and regulation of anti-Sm B cells, we generated 2-12H-Tg MRL/lpr mice. The Tg MRL/lpr mice used here are of the sixth or greater backcross generation. Like adult 2-12H-Tg mice (18), adult 2-12H-Tg MRL/lpr mice have anti-Sm B cells in the spleen (Fig. 1), although at a lower frequency (17.8 vs 29.8%; p = 8.1 × 10⁻⁵) (Table I). The frequency of anti-Sm B cells in young 2-12H-Tg mice of both nonautoimmune and autoimmune backgrounds is substantially greater than in their adult Tg counterparts. At 7 days of age, 53.5% of B cells are anti-Sm in nonautoimmune 2-12H-Tg mice, and 52.4% are anti-Sm in 2-12H-Tg MRL/lpr mice (Fig. 1 and Table I). A similar result was obtained at 21 days (data not shown). Thus, the proportion of anti-Sm B cells in 2-12H-Tg mice changes substantially as mice age, regardless of whether they are of a nonautoimmune or autoimmune genetic background.

Flow cytometry

Spleens and lymph nodes were collected and made into single-cell suspensions by crushing between the frosted ends of glass slides. All cells were prepared and washed in RPMI 1640 medium (HyClone, Logan, UT) containing 0.1% sodium azide and 3.0% bovine calf serum (HyClone). Following lysis of RBC using 0.15 M NH₄Cl, 1.0 mM KHCO₃, and 0.1 mM EDTA (pH 7.2–7.4), cells were washed, and staining was conducted at 4°C. Cells were stained in RPMI 1640 medium containing 0.1% sodium azide and 3.0% bovine calf serum with anti-IgM-FITC, anti-IgM-FITC, anti-CD24-FTTC, (heat-stable Ag; HSA), anti-CD19-PE, anti-B220-PE (CD45), and anti-CD23-FTTC purchased from BD PharMingen. Staining with biotinylated Sm followed by streptavidin-PerCP (BD PharMingen) to detect the autoreactive B cells was done as described previously (19). The cells were analyzed using a FACSscan (BD Biosciences, San Jose, CA) with hardware interface and acquisition and analysis software from Cytomation (Fort Collins, CO). All data represent cells that fell within the lymphocyte gate determined by forward and 90° light scatter. All contour plots are 5% probability.

Labeling with 5-bromo-2’-deoxyuridine (BrdU)

Adult mice were BrdU-labeled in vivo using the method of Allman et al. (24). Briefly, BrdU (Sigma) was administered in drinking water at 0.5 mg/ml with 1 mg/ml dextrose (Mallinckrodt, Paris, KY). After continuously labeling for 7 days, mice were sacrificed and spleen cells prepared for staining with anti-HSA-PE and anti-B220-CyChrome as described above. Subsequent permeabilization followed by treatment with DNase (Sigma) and staining with anti-BrdU-FTTC (BD Biosciences) allowed use of FACS analysis to measure the fraction of BrdU-labeled B cells (B220⁺).

FIGURE 1. Sm binding by B cells of autoimmune MRL/lpr and nonautoimmune 2-12H-Tg mice and their non-Tg littermates. The plots are from mice at 7 days of age and from adults (>3 mo) and are gated on CD19⁺ cells. The boxes indicate Sm-binding B cells used for quantification in Table I.

Non autoimmune

MRL/lpr

Non-Tg

Tg

100%

100%

51.4%

13.0%

52.9%

7.8%

24.4%

16.2%

26.1%

5.1%

Non autoimmune

MRL/lpr

Non-Tg

Tg

100%

100%

51.4%

13.0%

52.9%

7.8%

24.4%

16.2%

26.1%

5.1%

Downloaded from http://www.jimmunol.org/ by guest on September 7, 2017
of each genotype were analyzed. The contour plots are gated on CD19
from that with IgM. All mice are adults (3–5 mo of age). At least five mice
cells.

FIGURE 2. Phenotypic analysis of splenic B cells from 2-12H-Tg
MRL/lpr mice. Comparison is with splenocytes of a non-Tg MRL/lpr
littermate and a nonautoimmune 2-12H-Tg mouse. The brightest Sm-binding
cells were specifically boxed to the exclusion of the weaker binders.
Thus, the percentage of Sm-binding cells indicated is lower than it is in
Table I and Fig. 1. This was done to illustrate that the percentage of
Smbright cells in the histograms with HSA, CD23, and CD5 are not different
from that with IgM. All mice are adults (3–5 mo of age). At least five mice
of each genotype were analyzed. The contour plots are gated on CD19+ cells.

It is noteworthy that there are significant numbers of cells in the
Sm-binding gate in non-Tg mice (Table I and Fig. 1). Some of these cells may not be Sm-binding cells, because the trailing edge of
the negative population clearly overlaps the leading edge of the
positive population, making exclusion of some negative cells dif-
ficult. However, some cells in the Sm-binding gate are not stained
positive population, making exclusion of some negative cells dif-
Table I. Anti-Sm B cell differentiation in MRL/lpr mice

<table>
<thead>
<tr>
<th>7 Days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL/lpr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>% B cells (IgM+)</td>
<td>% Anti-Sm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Days Tg</td>
<td>3</td>
<td>3.0 ± 0.61</td>
<td>52.4 ± 2.20</td>
<td></td>
</tr>
<tr>
<td>7 Days Tg</td>
<td>3</td>
<td>3.9 ± 0.59</td>
<td>19.0 ± 1.30</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>2.45 × 10^-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tg</td>
<td>5</td>
<td>21.4 ± 6.6</td>
<td>17.8 ± 2.83</td>
<td></td>
</tr>
<tr>
<td>Tg</td>
<td>5</td>
<td>35.9 ± 5.8</td>
<td>7.14 ± 3.73</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>5.87 × 10^-3</td>
<td>4.80 × 10^-4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Nonautoimmune | | | | |
| n | % B cells (IgM+) | % Anti-Sm | p* |
| 6 | 9.07 ± 1.77 | 53.5 ± 2.2 | 0.570 |
| 2 | 6.90 ± 1.70 | 12.5 ± 0.8 | 7.91 × 10^-3 |
| 7 | 0.137 | 3.32 × 10^-7 | |
| 18 | 52.8 ± 12.9 | 29.8 ± 4.3 | 8.10 × 10^-4 |
| 18 | 63.4 ± 9.20 | 6.6 ± 3.2 | 0.526 |

* Comparison of the percentage of anti-Sm B cells between MRL/lpr and nonautoimmune mice.

It is noteworthy that there are significant numbers of cells in the
Sm-binding gate in non-Tg mice (Table I and Fig. 1). Some of these cells may not be Sm-binding cells, because the trailing edge of
the negative population clearly overlaps the leading edge of the
positive population, making exclusion of some negative cells dif-
ficult. However, some cells in the Sm-binding gate are not stained
positive population, making exclusion of some negative cells dif-
 frequency. The higher proportion of anti-Sm B cells in non-Tg
MRL/lpr mice relative to nonautoimmune mice may indicate a
predisposition in mice of the former strain to generate anti-Sm B
cells at a higher frequency.

Anti-Sm B cells in spleen and peritoneum

The majority of splenic anti-Sm B cells of 2-12H-Tg mice have a
transitional B cell phenotype; most are IgMhigh, HSAhigh, CD23-, CD43-, and CD5+ (Ref. 18 and Fig. 2). Flow cytometry analysis
of anti-Sm B cells from 2-12H MRL/lpr mice indicates that
anti-Sm B cells are also transitional in MRL/lpr mice. They are
IgMhigh, HSAhigh, CD5+, CD43+, and mostly CD23- (Fig. 2 and
data not shown). Some anti-Sm B cells in both nonautoimmune and autoimmune Tg mice are CD23+, and thus may be mature
B-2 cells.

Approximately 30% of peritoneal B-1 cells in nonautoimmune
2-12H-Tg mice are anti-Sm (20). In 2-12H-Tg MRL/lpr mice, the
percentage of peritoneal anti-Sm B cells is smaller than in their
nonautoimmune counterparts (6.7 vs 23.4%), but because there are
more B cells in MRL/lpr mice peritoneums, the total number of
anti-Sm B cells is not different (Table II). However, contrary to
those in nonautoimmune mice, the anti-Sm B cells of 2-12H-Tg
MRL/lpr mice do not appear to be B-1. They do not express CD5
or CD43 (Fig. 3), and they are smaller and less granular than nor-
mal B-1 cells (data not shown). In some mice, they express low
levels of CD23 (Fig. 3), but in most they are CD23- (data not
shown). Thus, the peritoneal anti-Sm B cells of nonautoimmune
and autoimmune MRL/lpr mice are phenotypically different.

Activation markers are up-regulated on anti-Sm B cells of
nonautoimmune mice

Because Ag selection is required for inclusion in the B-1 subset
(25–27) and exclusion of anti-Sm B cells from the B-2 subset is Ag
specific (19), we sought evidence that the anti-Sm B cells in the
spleen and peritoneum have encountered Ag. The levels of four
activation markers, CD40, CD44, and CD80, were mea-
sured on B cells of Tg and non-Tg mice (Fig. 4). The splenic
anti-Sm B cells of nonautoimmune 2-12H-Tg mice express higher
levels of CD40, CD44, and CD80 than do either non-Sm-binding
B cells of 2-12H-Tg mice or transitional B cells of non-Tg litter-
mate mice. For comparison, the transitional B cells of littermate
mice are defined as CD19+, CD23- (Fig. 4), but the same results
were obtained by using CD19+, HSAhigh B cells (data not shown).
CD86 levels are higher on anti-Sm B cells than on non-Sm binding
B cells of 2-12H-Tg mice, but not higher than on non-Tg tran-
sitional B cells. Also, some non-Tg transitional B cells have high
levels of CD40 and CD80 (Fig. 4), indicating that the high level
expression is not unique to anti-Sm B cells of Tg mice. Thus,
splenic anti-Sm B cells exhibit evidence of Ag stimulation.
Up-regulation of activation markers is also seen on peritoneal anti-Sm B cells. CD40, CD44, and CD86 are all expressed at higher levels on anti-Sm B cells than on non-Sm-binding peritoneal B cells of 2-12H-Tg mice or B-1 cells (defined as CD19<sup>+</sup>, CD23<sup>−</sup> cells) of non-Tg littermate mice. CD80 expression is not different between these B cell populations. Thus, both the splenic and peritoneal anti-Sm B cells of 2-12H-Tg mice show evidence of activation, indicating that they have encountered Ag.

**Activation markers are up-regulated on anti-Sm B cells of autoimmune MRL/lpr mice**

The splenic anti-Sm B cells of 2-12H-Tg MRL/lpr mice have higher levels of CD44, CD80, and CD86 than both non-Sm-binding B cells of Tg MRL/lpr mice and transitional B cells of non-Tg MRL/lpr littermates (Fig. 4). There is no apparent increase in CD40 expression. The level of CD80 and CD86 expression by a subset of anti-Sm B cells is substantially higher than on others. An equivalent population of anti-Sm B cells is not evident in nonautoimmune Tg mice.

CD86 and CD44 expression by peritoneal anti-Sm B cells of 2-12H-Tg MRL/lpr mice is higher than on non-Sm binding B cells of these mice, although they match the levels on some B cells of non-Tg MRL/lpr mice (Fig. 4). Also, essentially all anti-Sm B cells of Tg MRL/lpr mice express CD80, whereas only a subset of non-Sm-binding B cells of Tg MRL/lpr mice and B cells of non-Tg mice express CD80, suggesting that this marker is also up-regulated on these cells. In contrast, CD40 levels are not different between these cell populations. Thus, peritoneal anti-Sm B cells of MRL/lpr mice have up-regulated some activation markers, indicating Ag encounter.

The 2-12H transgene accelerates the spontaneous anti-Sm response in MRL/lpr mice and increases its prevalence

Little IgM<sup>+</sup> is detected in the sera of 2-wk-old 2-12H-Tg MRL/lpr mice, but, by 1 mo of age, all Tg mice have serum IgM<sup>+</sup> titers >2000 (Fig. 5A). In contrast, non-Tg mice have Ab titers by 2 wk of age. Thus, the 2-12H transgene suppresses the production of circulating Ab in very young mice, presumably because a substantial amount would be autoreactive (see paragraph below). Production of endogenous IgM<sup>+</sup> in Tg mice is largely undetectable until after 1 mo (Fig. 5A).

The levels of serum anti-Sm Abs in 2-12H-Tg mice are generally not different from those of non-Tg mice (19). However, in 2-12H-Tg MRL/lpr mice, elevated levels of anti-Sm Abs are present. Serum anti-Sm titers in more than half of 2-12H MRL/lpr mice tested were ≥1000 by 1 mo, and all were anti-Sm positive by 3 mo of age (Fig. 5B). Anti-Sm encoded by endogenous V genes, present as either IgM<sup>+</sup> or IgG, is undetectable (data not shown), and none of the non-Tg littermates tested at this age had detectable anti-Sm titers (Fig. 5B). Thus, the anti-Sm response is accelerated and the prevalence is increased to 100% in 2-12H-Tg MRL/lpr mice relative to non-Tg MRL/lpr mice.

**Overexpression of bcl-2 has no discernable effect on the phenotype or regulation of anti-Sm B cells**

In addition to MRL/lpr mice, anti-Sm Ab has been detected in mice overexpressing the anti-apoptosis gene bcl-2 (15). Moreover, the enforced expression of bcl-2 affects the regulation of other autoreactive B cells including inhibiting the death of autoreactive B-2 and B-1 cells (17, 18), although its overexpression has little effect on some anti-DNA B cells (20). To determine whether bcl-2 overexpression affects anti-Sm differentiation, we combined the 2-12H transgene with the bcl-2 transgene. The bcl-2 transgene is under the regulation of the H chain enhancer and promoter, and therefore its expression is restricted to B cells (15). Although bcl-2 is normally expressed in pro-B cells of the bone marrow and mature B cells and down-regulated in pre-B cells or immature/transitional B cells (28), it is expressed in B lineage cells at all stages in bcl-2-Tg mice (15).
All splenic B cells of 2-12H/bcl-2-Tg mice, including the transitional B cells, express the bcl-2 transgene, as determined by cytoplasmic staining (data not shown). The bcl-2 transgene has no significant effect (p > 0.05) on the total number of splenic B cells or on the number of splenic anti-Sm B cells in 2-12H-Tg mice (Table I). Also, the majority of splenic anti-Sm B cells of 2-12H/bcl-2-Tg mice are IgM+IgGa high, HSA high, and CD23+ (Fig. 6A), and CD5+ and CD43+ (data not shown), a transitional B cell phenotype. Consistent with this, their turnover rate, as determined by BrdU incorporation, is short; >50% of the anti-Sm B cells are BrdU+ in the 2-12H/bcl-2 double-Tg mice after 7 days of BrdU treatment, vs 40% in 2-12H-Tg mice (Fig. 6C). Anti-Sm B cells are not detectable in the lymph nodes or bone marrow (data not shown). In the peritoneum, the anti-Sm B cells have a B-1 phenotype, i.e., IgM+IgGa high, CD23−, CD5+ (Fig. 6B), and CD43− (data not shown). Finally, the levels of anti-Sm in circulation are not different from those of non-Tg and 2-12H-Tg mice (thin line). The Sm-binding cells gated in this analysis are the Sm-brightest population indicated by the gates in Figs. 2 and 3.

**Discussion**

This study examines the differentiation of autoreactive anti-Sm B cells in nonautoimmune and autoimmune 2-12H-Tg MRL/lpr mice. Expression of each marker by spleen cells is indicated separately for anti-Sm B cells of Tg mice (bold line), non-Sm-binding transitional B cells of Tg mice (dotted line), and transitional B cells of non-Tg mice (thin line). Expression by peritoneal B cells is indicated for anti-Sm B cells of Tg mice (bold line), non-Sm-binding B cells of Tg mice (dotted line), and B-1 cells from non-Tg mice (thin line). The Sm-binding cells gated in this analysis are the Sm-brightest population indicated by the gates in Figs. 2 and 3.

**Table III. Splenic B cells of 2-12H and 2-12H/bcl-2-Tg mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>n</th>
<th>No. of B Cells (× 10^7)</th>
<th>% B Cells</th>
<th>No. of Anti-Sm B Cells (× 10^7)</th>
<th>% Anti-Sm B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-12H/bcl-2 Tg</td>
<td>6</td>
<td>6.4 ± 2.3</td>
<td>44.5 ± 9.8</td>
<td>1.8 ± 0.63</td>
<td>29.5 ± 8.0</td>
</tr>
<tr>
<td>2-12H</td>
<td>6</td>
<td>4.1 ± 1.1</td>
<td>37.7 ± 5.5</td>
<td>1.1 ± 0.43</td>
<td>25.1 ± 6.0</td>
</tr>
<tr>
<td>Non-Tg</td>
<td>6</td>
<td>9.8 ± 3.9</td>
<td>55.1 ± 16.3</td>
<td>0.60 ± 0.25</td>
<td>6.2 ± 1.9</td>
</tr>
</tbody>
</table>

and increases the prevalence of the anti-Sm response in 2-12H-Tg MRL/lpr mice. Moreover, anti-Sm B-1 cells are absent from MRL/lpr mice, suggesting that differentiation to B-1 may be important to maintaining tolerance to Sm.

The high frequency of anti-Sm B cells in neonatal mice of both nonautoimmune and nonautoimmune backgrounds suggests that the majority of newly generated B cells are anti-Sm. We presume that the L chain repertoire is diverse in early life, and, therefore, that the high frequency of anti-Sm B cells is due to the ability of the 2-12H chain to pair with diverse L chains and bind Sm. This would be consistent with our in vitro analysis of Sm binding by 2-12H chain Abs in which Abs composed of 2–12 H chains paired with different L chains (at least five of nine tested) bind Sm (29). In contrast, most B cells in adult mice of both strains do not bind Sm (Fig. 1, Table I, and Ref. 19)). Because anti-Sm B cells appear to be selectively excluded from the mature B-2 population, the change in frequency of anti-Sm B cells as mice age is probably due to the gradual accumulation of non-Sm-binding B cells in the maturity B-2 subset (19). This ability to generate mostly autoreactive B cells is a feature this transgene shares with the anti-DNA 3H9 H chain transgene (4).

The encounter of anti-Sm B cells with Ag, indicated by the increase in activation marker expression, likely induces multiple outcomes: 1) Some anti-Sm B cells may be driven to become mature B-1 cells as a result of Ag encounter (20). Anti-Sm B cells account for ~30% of the peritoneal B-1 repertoire in 2-12H-Tg mice, and splenic anti-Sm B cells transferred from 2-12H-Tg mice to irradiated littermates differentiate to B-1 (20). However, despite the capability of differentiation to B-1, it is unlikely that all transitional anti-Sm B cells normally differentiate to B-1 in adult mice,
because there is little differentiation to B-1 from adult bone marrow in normal mice (30). Moreover, the number of anti-Sm transitional B cells in the spleens of 2-12H-Tg mice exceeds by 30-fold the number of anti-Sm B-1 cells in the peritoneum, arguing that many of these transitional B cells do not normally differentiate to B-1. 2) Many transitional anti-Sm B cells may undergo programmed cell death as a result of Ag encounter. We have proposed this previously (19), because most anti-Sm B cells are excluded from the B-2 subset and probably do not differentiate to B-1 (20). 3) Some anti-Sm B cells may differentiate to mature B-2 cells because some of these cells express CD23 (19). A recent analysis of low affinity anti-Sm B cells indicates that they differentiate to mature B-2 cells but are anergic (M. Borrero and S. H. Clarke, manuscript in preparation). The distinction between those cells that undergo cell death and of those that differentiate to B-2 or B-1 may be affinity for Ag. We have begun to address the role of affinity for Sm by combining the 2-12H transgene with various L chain transgenes. Thus, the outcome of transitional anti-Sm B cell encounter with Ag likely includes differentiation to mature B-2 cells but are anergic (M. Borrero and S. H. Clarke, manuscript in preparation). The junction between those cells that undergo cell death and of those that differentiate to B-1 or B-2 may be affinity for Ag. We have begun to address the role of affinity for Sm by combining the 2-12H transgene with various L chain transgenes. Thus, the outcome of transitional anti-Sm B cell encounter with Ag likely includes differentiation to mature B-2 cells but are anergic (M. Borrero and S. H. Clarke, manuscript in preparation). The junction between those cells that undergo cell death and of those that differentiate to B-1 or B-2 may be affinity for Ag. We have begun to address the role of affinity for Sm by combining the 2-12H transgene with various L chain transgenes. Thus, the outcome of transitional anti-Sm B cell encounter with Ag likely includes differentiation to mature B-2 cells but are anergic (M. Borrero and S. H. Clarke, manuscript in preparation).

Enforced bcl-2 expression does not appear to affect anti-Sm B cell differentiation or serum anti-Sm levels in 2-12H/bcl-2-Tg mice. This is different from our expectation based on the anti-Sm secretion observed in the original Em/bcl-2-Tg mice (15) and the effect of bcl-2 expression on peripheral B cell deletion (16–18). The presence of serum anti-Sm in the original Em/bcl-2-Tg mice is probably due to their autoimmune SJL genetic background. The mice used here have a C57BL/6 (B6) background and therefore lack the genes that predispose to autoantibody production. Nevertheless, enforced bcl-2 expression affects the peripheral regulation of B cells specific for membrane Ags and can inhibit cell death of peritoneal B-2 and B-1 cells upon exposure to self-Ag (17, 18). Thus, multiple factors are involved in the role of bcl-2 in tolerance, and these data indicate that regulated bcl-2 expression is not required for maintaining tolerance to Sm. This is similar to that observed for anti-DNA B cells in nonautoimmune mice (31).

Tolerance to Sm is lost in 2-12H-Tg MRL/lpr mice as the 2-12H transgene accelerates and increases the prevalence of the spontaneous anti-Sm response in MRL/lpr mice. The autoimmune disease of MRL/lpr mice is caused by multiple genetic loci (32–34), in addition to the lpr mutation, and the defect for autoantibody production is intrinsic to both B and T cells (35–38). The underlying mechanism for anti-Sm B cell activation is unlikely to be affected by the H chain transgene, and, thus, the transgene effect must be through its ability to increase the number of anti-Sm B cells. Because the anti-Sm response in MRL/lpr mice is the product of a stochastic event occurring in individual mice (11), we suggest that the higher frequency of anti-Sm B cells increases the probability that this event occurs in all mice and at an earlier age. Thus, the number of anti-Sm B cells in MRL/lpr mice is the limiting factor in the low prevalence of this response. Interestingly, the increase in the number of anti-DNA B cells in 3H9-Tg MRL/lpr mice has no effect on the age of onset of the anti-DNA response, suggesting that anti-DNA B cell numbers are not limiting (39). Also, this difference argues that the underlying mechanism responsible for activation of anti-Sm and anti-DNA B cells is different, consistent with differences in their regulation in nonautoimmune mice (4, 19, 20, 40) and in the prevalence of these responses in MRL/lpr mice (1).
The differentiation of anti-Sm B cells is altered in MRL/lpr mice consistent with the high levels of serum anti-Sm. Differentiation to the transitional B cell stage in 2-12H-Tg MRL/lpr mice is intact, and these cells up-regulate activation markers. Intriguingly, some anti-Sm B cells express CD80 and CD86 at levels 2- to 5-fold higher than other anti-Sm B cells. This subset is not seen in non-autoimmune Tg mice. CD80 and CD86 provide vital signals for the generation of T cell responses through interaction with CD28 and CTLA-4 (41–45). Blockade of CD80 and CD86 function with Abs interferes with the development of anti-Sm RNP responses in MRL/lpr mice (46, 47), and blockade of just one or the other molecule, while not preventing the secretion of autoantibodies, can alter the severity of kidney disease (47). MRL/lpr mice deficient in both CD80 and CD86 exhibit little disease (46). Thus, the anti-Sm B cells expressing high levels of CD80 and CD86 may be cells actively engaged in the anti-Sm response. We are currently testing this possibility.

The most striking difference between autoimmune and nonautoimmune 2–12-H-Tg mice is in the peritoneum. Whereas ~30% of peritoneal B-1 cells are anti-Sm in 2-12-H-Tg mice, few or none of the peritoneal anti-Sm B cells of 2-12-H-Tg MRL/lpr mice are B-1 (Fig. 3). The loss of B-1 cells is not unique to anti-Sm B cells, as non-Sm-binding B-1 cells are also absent from 2-12-H-Tg MRL/lpr mice and from non-Tg MRL/lpr littermates (Fig. 3). The absence of peritoneal B-1 cells was originally observed in B6/lpr mice by Reap et al. (48), implicating the lpr gene in the loss of these cells. In B6/lpr mice, peritoneal B-1 cells develop in early life but disappear at ~3 mo of age (48). A cytokine imbalance is suggested to be the cause for the B-1 cell defect (48). The peritoneal anti-Sm B cells of 2-12-H-Tg MRL/lpr mice resemble transitional B cells because they are IgMgh and usually CD23− (Fig. 3 and (49)). However, some express low levels of CD23 (CD23low) and others have higher levels more typical of a mature B-2 cell (CD23+; data not shown). CD23low B cells were previously noted in the peritoneums and spleens of MRL/lpr and B6/lpr mice (50). They may be transitional B cells, which can express CD23 (49), or they may be B-1 cell precursors. Our analysis of the differentiation of anti-phosphatidyl choline B cells to B-1 indicates that precursors to B-1 in the spleen express CD23 (51). Regardless of the differentiative stage of these cells, the absence of peritoneal B-1 cells suggests that the block in B-1 cell development may contribute to the loss of tolerance to Sm.

An important question raised by the disruption in B-1 cell survival or differentiation in MRL/lpr mice is what happens to anti-Sm B cells that would otherwise differentiate to B-1. One possibility is that they are arrested in development at the mature B-2 cell stage. Our analysis of the differentiation of phosphatidyl choline-specific B cells indicates that B-2 cells are precursors to B-1 (26, 51). Similarly, in 2-12H-Tg mice that lack CD19, which are deficient in B-1 cell differentiation, splenic and peritoneal anti-Sm B cells are B-2 rather than B-1 (20). Because B cells are positively selected for entry into the B-1 subset, a block in B-1 cell development could lead to the accumulation of activated and functional anti-Sm B-2 cells. T cell tolerance to Sm is lost in MRL/lpr and 2-12H-Tg MRL/lpr mice (52), and, thus, activated anti-Sm B cells could be drawn into a T cell-dependent response. Other pathways of activation could occur as well. For example, anti-Sm B cell anergy or their developmental block at the transitional B cell stage could be broken, as demonstrated for anti-DNA B cells (39, 53). We are currently examining the possibility that the block in B-1 cell differentiation contributes to the loss of tolerance to Sm.

In summary, anti-Sm B cell differentiation to B-1 is blocked in autoimmune 2-12-H-Tg MRL/lpr mice, and the 2-12H transgene decreases the time of onset of the anti-Sm response and increases its prevalence in these mice. These observations differ from those of B cells specific for the lupus Ags DNA and rheumatoid factor in Ig-Tg mice (6, 39, 53). These cells do not differentiate to B-1 in nonautoimmune mice. Instead, anti-ssDNA B cells acquire a mature B cell phenotype and are regulated by anergy (40), while anti-dsDNA B cells are developmentally blocked at the transitional B cell stage (4). Anergy to ssDNA and the developmental block of anti-dsDNA B cells are broken in MRL/lpr mice (39, 53). In contrast, rheumatoid factor B cells are regulated in nonautoimmune mice by ignorance (54), but are activated in Fas-deficient autoimmune mice (6). Thus, lupus-specific B cells are regulated by diverse mechanisms. This may mean that there are multiple defects in autoimmune MRL/lpr mice, each affecting B cells of a different differentiative pathway. Alternatively, there may be a single defect that is manifested differently in each of the differentiative pathways followed by lupus Ag-specific B cells.

References


