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Separation of the New Zealand Black Genetic Contribution to Lupus from New Zealand Black Determined Expansions of Marginal Zone B and B1a Cells

Stephanie Atencio,*† Hirofumi Amano,‡ Shozo Izui,‡ and Brian L. Kotzin2*†

The F1 hybrid of New Zealand Black (NZB) and New Zealand White (NZW) mice develop an autoimmune disease similar to human systemic lupus erythematosus. Because NZB and (NZB × NZW)F1 mice manifest expansions of marginal zone (MZ) B and B1a cells, it has been postulated that these B cell abnormalities are central to the NZB genetic contribution to lupus. Our previous studies have shown that a major NZB contribution comes from the Nba2 locus on chromosome 1. C57BL/6 (B6) mice congenic for Nba2 produce antinuclear Abs, and (B6.Nba2 × NZW)F1 mice develop elevated autoantibodies and nephritis similar to (NZB × NZW)F1 mice. We studied B cell populations of B6.Nba2 mice to better understand the mechanism by which Nba2 leads to disease. The results showed evidence of B cell activation early in life, including increased levels of serum IgM, CD69+ B cells, and spontaneous IgM production in culture. However, B6.Nba2 compared with B6 mice had a decreased percentage of MZ B cells in spleen, and no increase of B1a cells in the spleen or peritoneum. Expansions of these B cell subsets were also absent in (B6.Nba2 × NZW)F1 mice. Among the strains studied, B cell expression of β2 integrin correlated with differences in MZ B cell development. These results show that expansions of MZ B and B1a cells are not necessary for the NZB contribution to lupus and argue against a major role for these subsets in disease pathogenesis. The data also provide additional insight into how Nba2 contributes to lupus. The Journal of Immunology, 2004, 172: 4159–4166.

Systemic lupus erythematosus (SLE)3 is an autoimmune disease characterized by elevated levels of IgG antinuclear autoantibodies and the development of an immune complex-mediated glomerulonephritis (1, 2). New Zealand Black (NZB) and New Zealand White (NZW) F1 hybrid mice spontaneously develop similar autoimmune abnormalities and are considered to be an excellent mouse model of the human disease (3). (NZB × NZW)F1 parent and NZB mice also demonstrate expansions of marginal zone (MZ) B cells, localized at the junction of white and red pulps in spleen, and expansions of B1a cells in both spleen and peritoneum (4–10). These B cell subset expansions are present in young (pre-disease) NZB mice and are associated with other intrinsic B cell defects in NZB mice, including spontaneous polyclonal B cell activation and B cell hyperreactivity (11–17). Primarily based on the association of expansions with the development of lupus, the MZ B and B1a subsets have been postulated to be critically involved in the NZB genetic contribution to disease in (NZB × NZW)F1 and other NZB-dependent lupus models (4–10). B1a and MZ B cells have also been shown to be enriched for autoreactive specificities and responsible for IgM autoantibody production in certain experimental situations (18–21). It remains to be established, however, whether B1a or MZ B cells secrete pathogenic IgG autoantibodies in the lupus models. Evidence against the idea that MZ B and B1a cells are important in lupus includes the fact that other lupus-prone strains do not have associated expansions of these subsets (3, 7, 22). In fact, in a recent study of Yaa-associated B cell abnormalities (26). One way to address the importance of a strain’s various traits in the development of lupus is through isolation of individual genetic contributions. We previously mapped an important NZB lupus-susceptibility locus, named Nba2 (for NZB autoimmunity 2), to the NZB genetic contribution to disease, when tested in the context of the B6 and NZW backgrounds. B6.Nba2 congenic mice were crossed to NZW mice, the F1 progeny developed autoantibody production and lupus nephritis in a pattern nearly identical with (NZB × NZW)F1 mice (26). The similarity in disease phenotype between (B6.Nba2 × NZW)F1 and (NZB × NZW)F1 implicated Nba2 as the major NZB genetic contribution to disease, when tested in the context of the B6 and NZW backgrounds. B6.Nba2, compared with B6 mice, also showed increased spleen cell numbers, which included both total B cell and T cell populations, and additional studies suggested that Nba2 might be associated with B cell abnormalities (26).

The present study was designed to test whether NZB B cell activation and NZB MZ B and B1a cell expansions are encoded within Nba2 and associated with this genetic contribution to lupus. The results show that B6.Nba2 mice manifest spontaneous B cell hyperactivity early in life. However, these mice lack MZ B cell expansions in the spleen and lack B1a cell expansions in the spleen and peritoneum. In fact, the percentage of MZ B cells in B6.Nba2

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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; MZ, marginal zone; NZB, New Zealand Black; NZW, New Zealand White.

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mice was significantly lower than in B6 mice, which was mirrored by a decrease in B cell expression of βλ integrin. The results suggest that these expansions may just be an interesting trait of the NZB strain rather than a necessary part of its contribution to lupus. Together with other studies (22), our data argue against a role for MZ B and B1a cells in the generation of pathogenic autoantibodies in mouse lupus.

Materials and Methods

Mice

C57BL/6 (B6), NZB/NBIJ, NZW/LacJ, and (NZB × NZW)F1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B6.NZB-Nba2 (designated B6.Nba2) mice containing a 30-cM interval derived from distal NZB chromosome 1 were derived as previously described (26) and used to breed (B6.Nba2 × NZW)F1 mice. Concomitantly bred (B6 × NZWF1) mice served as controls. All mice studied were female. These mouse studies were approved by the Animal and Care Use Committee of the University of Colorado Health Sciences Center (Denver, CO).

Cell preparation and flow cytometric analysis

Spleenic mononuclear cells were obtained by passage of spleen through a wire mesh or agitation between two glass microscope slides, washing in HBSS (Life Technologies, Grand Island, NY), and RBC lysis with hypotonic ammonium chloride. The remaining cells were washed again in HBSS and resuspended in PBS containing 5% FBS (Life Technologies) for surface staining. Peritoneal lymphocytes were obtained by peritoneal lavage with 5 ml of cold HBSS. These cells were also resuspended in PBS containing 5% FBS before staining.

Flow cytometry was performed using three-color staining of spleen or peritoneal cells. Staining reagents included mAbs to B220, CD19, CD5, CD23, CD43, CD21, Mac-1 (CD11b), B6, B7-1 (CD80), B7-2 (CD86), CD1d, LFA-1α-chain (CD11a), and integrin βλ-chain (CD29) (all from BD PharMingen, San Diego, CA). Spleen cells were incubated at 1 × 10⁶ cells/well in 96-well plates in staining solution containing saturating levels of Fc receptor-blocking Abs (goat anti-mouse CD16/CD32. BD Biosciences, Mountain View, CA) for 5 min at room temperature before the staining mAbs were added. Cells were stained in PBS containing 5% FBS on ice for 30 min. After washing, immunofluorescence staining was analyzed with a FACScan (BD Biosciences). Scatter gates were set to include the viable lymphocyte population, and 30,000–50,000 events were collected for each sample.

Measurement of serum IgM by ELISA

Total IgM levels in sera from 8-wk-old female mice were analyzed by ELISA as described (24). Briefly, wells of Immunul II microtiter plates (Dynatech Laboratories, Alexandria, VA) were coated with 2.5 μg/ml goat anti-mouse IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Plates were washed with PBS and blocked for 1 h at 37°C with PBS-gelatin. Sera were diluted 1/10,000 and 1/20,000 in PBS with 5% FBS, and 100 μl of diluted serum was added to each well and incubated for 1 h at room temperature. After washing in PBS, a secondary peroxidase-conjugated goat anti-mouse IgM (Kirkegaard & Perry Laboratories) was added for 1 h at room temperature. After washing and the addition of substrate, OD was read on a Vmax kinetic microplate reader and analyzed via Softmax (Molecular Devices, Sunnyvale, CA). A standard curve was generated using serial dilutions of a polyclonal mouse IgM (Kirkegaard & Perry Laboratories), and values were converted to microgram per milliliter as described (24). All assays were performed in triplicate.

Spontaneous IgM production in vitro

B cell-enriched spleen cells were prepared by two different techniques. In the first, spleen cells were depleted of T cells using anti-Thy-1 (clone T24, provided by Dr. J. Cambier, National Jewish Medical and Research Center, Denver, CO) followed by addition of rabbit complement (Pel-Freez Clinical Systems, Brown Deer, WI). Remaining CD3+ cells were <3% of the lymphocyte population. In other experiments, isolation of nonactivated B cells was accomplished by depletion of CD43+ cells, using anti-CD43 microbeads followed by automated magnetic cell sorting (AutoMACS; Miltenyi Biotec, Bergisch Gladbach, Germany). Either purification technique resulted in lymphocyte populations that were greater than 95% B220+ B cells. A total of 1 × 10⁶ viable cells were then cultured in 200 μl RPMI 1640 medium with 10% FBS in 96-well flat-bottom plates for 48 h. Culture supernatants were collected and analyzed for total IgM levels by ELISA as previously described.

Statistical analysis

The statistical significance of differences in IgM levels between strains was determined using the nonparametric Mann-Whitney U test. The Student t test was used to determine the statistical significance of differences in the percentage of particular B cell subsets between strains.

Results

Evidence for spontaneous B cell activation in young B6.Nba2 mice

NZB mice manifest elevated serum levels of total IgM early in life, well before the production of autoantibodies or development of disease (5, 11–15, 17). These elevated levels have been shown to reflect the early spontaneous B cell activation that occurs in NZB mice. We measured serum IgM levels in B6.Nba2 mice at 8 wk of age, before the development of autoantibodies, and compared levels to B6 and NZB mice. Fig. 1 shows that the mean levels in young B6.Nba2 mice were 5-fold greater and significantly higher compared with B6 mice (p = 0.0002). Levels in NZB mice were even more elevated (p = 0.02 compared with B6.Nba2 mice), suggesting that loci in addition to Nba2 contribute to this phenotype in the NZB strain.

In our analyses of B cell phenotype and various B cell subsets, expression of B220 and CD19 was used to identify the total B cell population. In the different strains studied, >99% of the gated B220+ cells in spleen and peritoneum also expressed CD19 (data not shown). In addition, nearly all CD19+ cells expressed high levels of B220 in B6.Nba2 and B6 mice in both spleen and peritoneum and the pattern of expression appeared similar (Table I). Compared with B6 mice, B6.Nba2 mice had a slightly lower percentage of splenic B cells but nearly 2-fold increased numbers of total spleen cells (Table I). A small population of CD19+ cells in NZB spleen appeared to have decreased B220 expression, suggesting that a small subset of B1a or activated B cells partially down-regulate B220 surface expression in this strain (see below).

We analyzed splenic B cells from young B6.Nba2 mice for the expression of the early activation marker, CD69 (Fig. 2). The percentage of B cells positive for CD69 was significantly higher in B6.Nba2 compared with B6 mice (7.2 ± 1.0 vs. 3.3 ± 0.14%; p < 0.001). The percentage of CD69+ B cells was even higher in the two NZB mice studied. In contrast to CD69, neither the level of CD80 or CD86 expression nor the percentage of CD80+ or CD86+ B cells was increased in B6.Nba2 compared with B6 mice (data not shown).

As a functional measure of B cell activation, T cell-depleted spleen cells or CD43-depleted spleen cells from 8-wk-old B6 and B6.Nba2 mice were cultured without deliberate activation, and

FIGURE 1. Serum IgM levels in 8-wk-old mice. Each symbol represents a different mouse studied and the lines indicate the mean level for each respective group. Levels in B6.Nba2 were significantly greater than B6 mice (p = 0.0002) and significantly less than NZB mice (p = 0.02).
spontaneous IgM production was quantified in the culture supernatants. Fig. 3 shows significantly increased IgM production by B cells from B6.Nba2 mice compared with B6 mice (p = 0.006 by paired Student’s t test). Both techniques of B cell enrichment resulted in similar production levels and similar differences between the two strains, and the data were therefore pooled. Consistent with serum IgM levels, NZB B cells demonstrated the highest levels of in vitro IgM production (p = 0.002 compared with B6.Nba2 B cells) (Fig. 3).

**Absence of MZ B cell expansion in B6.Nba2 mice**

Previous studies in NZB mice have shown that expansions of MZ B cells occur by 6–7 wk of age, before IgG autoantibody production (4, 5). To determine whether the Nba2 contribution to lupus also involves an expansion of these cells, we compared the percentage of MZ B cells in the spleens of 8-wk-old congenic B6.Nba2, B6, and NZB mice. Initial studies focused on the percentage of splenic B220+ cells that did not express CD5 and expressed negative or low levels of CD23. The expression of these Ags and the percentage of CD5\(^-\)CD23\(^{low}\) B cells in the spleens of B6.Nba2 and B6 mice were similar (Fig. 4A). In contrast, a marked decrease in the expression of CD23 was apparent in a subset of NZB B cells, which resulted in a 2- to 3-fold increase in the percentage of cells counted as CD23\(^{low}\). When multiple mice were compared, the mean percentage (±SEM) of CD5\(^-\)CD23\(^{low}\) B cells in B6 mice was 15.8 ± 0.7% compared with 4.6 ± 1.3% in the B6.Nba2 congenic (Fig. 4A). In contrast, the mean percentage of this subpopulation of B cells in NZB mice was 48.2 ± 4.3% (p < 0.001 compared with either B6 or B6.Nba2). No increase in this population was apparent as the animals aged (Fig. 4A).

MZ B cells were separated from other subsets in the CD5\(^-\)CD23\(^{low}\) B cell population, such as transitional 1 B cells, by relatively high expression of CD21 (4–6, 22, 27, 28). Representative staining patterns and results for different mice are shown in Fig. 4B. Interestingly, the percentage of CD23\(^{low}\)CD21\(^{high}\) cells in the B220\(^+\) population was significantly decreased in B6.Nba2 compared with B6 mice (2.6 ± 0.26% compared with 5.8 ± 0.42%; p < 0.001). In contrast, the percentage in NZB mice (mean ± SEM, 11.9 ± 1.8%) was significantly greater (p < 0.001 compared with either B6 or B6.Nba2). The reduction and increase of MZ B cells in B6.Nba2 and NZB mice, respectively, were further confirmed with the use of another marker, CD1d, which is highly expressed on MZ B cells (29), in combination with CD21 and CD23 staining (data not shown). We also examined older B6.Nba2 and B6 mice, ranging in age from 6 to 12 mo, to insure that there was not a delayed MZ B cell expansion in B6.Nba2 mice (Fig. 4B; open symbols). No increase in the percentage of MZ B cells occurred in older congenics or B6 controls.

In the course of these studies of MZ B cells, we also noted an increase in the percentage of splenic CD21\(^{low}\)CD23\(^{low}\) B220\(^+\) cells in B6.Nba2 compared with B6 mice (7.49 ± 2.5 vs 13.9 ± 0.7; p = 0.02) (Fig. 4B). The phenotype of these cells is characteristic of transitional 1 B cells (28).

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**Table 1. B cell percentages and numbers in the spleen of study mice**

<table>
<thead>
<tr>
<th>Strain (n)</th>
<th>Spleen Cell Count (× 10(^{-7}))</th>
<th>CD19(^+)B220(^{high}) (%); CD19(^+)B220(^{low}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6 (5)</td>
<td>5.2 ± 0.5</td>
<td>53.8 ± 3.8; 1.5 ± 0.1</td>
</tr>
<tr>
<td>B6.Nba2 (5)</td>
<td>10.4 ± 1.2</td>
<td>42.8 ± 2.8; 1.5 ± 0.1</td>
</tr>
<tr>
<td>NZB (6)</td>
<td>6.9 ± 0.5</td>
<td>30.0 ± 5.5; 6.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Cells from 8-wk-old mice were analyzed. Values are the mean percentage ± SEM of spleen cells.

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**Decrease in \(\beta_1\) integrin expression on B cells in B6.Nba2 mice**

We investigated potential mechanisms for the decreased development of MZ B cells in B6.Nba2 mice as well as the expansion of this subset in NZB mice. Recent studies have demonstrated that increased expression of integrins LFA-1 (\(\alpha_\text{L} \beta_2\)) and \(\alpha_\text{L} \beta_1\) on MZ B cells is likely involved in their localization and retention in the MZ (30). Staining with mAb to LFA-1 \(\alpha\text{-chain (CD11a) and } \beta_1\) integrin confirmed higher expression of these two integrins on MZ B cells (B220\(^+\)CD23\(^{low}\)CD21\(^{high}\)) compared with follicular B cells (B220\(^+\)CD23\(^{high}\)CD21\(^{low}\)) (Fig. 5A and data not shown). Interestingly, significant differences were noted in expression of \(\beta_1\) integrin on MZ B cells among the different strains (Fig. 5B). MZ B cells in NZB mice (mean fluorescence intensity ± SEM, 76.3 ± 2.0) expressed substantially greater levels than in B6 or B6.Nba2 mice (p < 0.001), whereas levels in B6.Nba2 mice (39.2 ± 0.94) were slightly, but significantly, lower than in B6 mice (49.7 ± 0.67) (p < 0.001). Thus, differences in expression of \(\beta_1\) integrin on MZ cells paralleled differences in the size of this subset in the three strains. Although expression levels of \(\beta_1\) integrin on follicular B cells were lower and differences among the three strains were less marked, these differences were also statistically significant (p < 0.001) with NZB highest and B6.Nba2 lowest (Fig. 5B).

In contrast to expression of \(\beta_1\) integrin, MZ and follicular B cell expression of LFA-1 \(\alpha\text{-chain was similar among these three strains of mice (mean fluorescence intensity for MZ B cells: NZB, 44.9 ± 0.6; B6.Nba2, 42.5 ± 1.4; B6, 45.9 ± 1.5) (follicular B cells: NZB, 21.7 ± 0.6; B6.Nba2, 16.1 ± 0.9; B6, 16.7 ± 0.5).
Normal percentages of B1a cells in the spleen and peritoneum of young B6.Nba2

Previous studies have also demonstrated expansions of B1a cells in both the spleen and peritoneal cavity of NZB mice (7–10). Like MZ B cells, the increased percentage of these cells has been postulated to be pivotal in the NZB contribution to lupus in (NZB × NZW)F1 mice. Splenic B cells from 8-wk-old mice were stained for the B1a surface phenotype, B220–CD5–CD23low. Representative staining patterns and results in multiple mice from each strain are shown in Fig. 6A. The results show no difference in percentage of B1a cells in the spleens of congenic B6.Nba2 compared with B6 mice (3.8 ± 0.58% vs 3.2 ± 0.39%) but a significantly elevated level (20.7 ± 2.1%) in NZB mice (p < 0.001 compared with either B6 or B6.Nba2). No expansion of the B1a population occurred in older B6 or B6.Nba2 mice (Fig. 6A).

As previously described, nearly all peritoneal CD19+ cells express high levels of B220 in all three strains studied. After gating on B220+ cells, we used expression of CD5 and Mac-1 (CD11b) to define the B1a cell population in the peritoneal cavity (10). The percentages of B220+ cells phenotyped as CD5+ and CD5– Mac-1+ were not increased in B6.Nba2 compared with B6 mice (Fig. 6B). In contrast, this population was greatly increased in NZB mice. Overall, we found 20.1 ± 1.9% B1a cells in the B6.Nba2 peritoneal cavities and 25.8 ± 4.5% B1a cells in B6 mice, which were not statistically different from each other. These results are consistent with previous reports of the levels of this B cell subset in the peritoneum (10). Similar to previous reports, NZB mice had much larger percentages of B1a cells with a mean level of 57.5 ± 4.0% (p < 0.001 compared with either B6 or B6.Nba2 mice).

(B6.Nba2 × NZW)F1 and (B6 × NZW)F1 have similar levels of MZ B and B1a cells

Expansions of MZ B and B1a cells are also well documented in (NZB × NZW)F1 mice (7–10). Our previous studies showed that the development of elevated IgG autoantibody levels and lupus nephritis was similar in (B6.Nba2 × NZW)F1 mice compared with (NZB × NZW)F1 mice, whereas (B6 × NZW)F1 mice had little if any evidence of lupus-like autoimmunity (26). We therefore used the same techniques as previously described to study the different F1 strains as well as parental NZB and NZW strains. Mice studied were 8 wk of age (Fig. 7; closed symbols), which is before the development of IgG autoantibody production and autoimmune manifestations, or 6- to 12-mo-old (Fig. 7; open symbols). The mean percentage of splenic CD5–CD23low B cells was comparable between (B6.Nba2 × NZW)F1 and (B6 × NZW)F1 mice, whereas there was a trend for higher levels in (NZB × NZW)F1 mice (p = 0.06 vs (B6 × NZW)F1 and p = 0.07 compared with (B6.Nba2 × NZW)F1; Fig. 7A). Levels of CD5–CD23low B cells in NZW were similar to B6 and B6.Nba2 mice and lower than in NZB mice (see Fig. 4A). CD21high/CD23low (MZ) B cells were also not different in young (B6.Nba2 × NZW)F1 compared with (B6 × NZW)F1 mice, which were both lower than age-matched (NZB × NZW)F1 mice (Fig. 7B). Interestingly, we noted a drop in the size of this subset as (NZB × NZW)F1 mice aged and in the one older (B6.Nba2 × NZW)F1 studied. The percentage of splenic MZ cells in NZW mice was similar to (B6 × NZW)F1 and (B6.Nba2 × NZW)F1 mice and lower than NZB mice.
In a similar manner, we analyzed the B1a population in the spleen (Fig. 7C) and peritoneum (Fig. 7D) of the different F1 mice. The percentages of B1a cells in the spleens of (B6.Nba2 × NZW)F1 and (B6 × NZW)F1 were similar (6.4 ± 0.6% and 6.4 ± 1.0%, respectively) and significantly lower than in (NZB × NZW)F1 mice (p = 0.01). Parental NZB (see Fig. 6) and NZW mice (Fig. 7) had even higher levels of this subset. Similar findings were apparent when we quantified the percentage of B1a cells in the peritoneum. Again, the level of B1a cells in (B6.Nba2 × NZW)F1 mice (28.0 ± 4.8%) was not higher than that in (B6 × NZW)F1 mice (35.1 ± 5.8%).

Discussion

The present studies addressed the relevance of MZ B and B1a cell expansions to autoantibody production and lupus-like disease in NZB and (NZB × NZW)F1 mice. The mapping of Nba2 and demonstrations of autoantibody production in congenic B6.Nba2 mice and similar disease in (B6.Nba2 × NZW)F1 vs (NZB × NZW)F1 mice (26) allowed us to begin to segregate phenotypes essential for the NZB contribution to disease. Our results show that B6.Nba2 mice, like NZB mice, demonstrate early B cell activation, as documented by increased IgM production both in vivo and in vitro and increased percentages of CD69+ B cells in spleen (3–5, 11–17). This was apparent at 8 wk of age, well before elevated levels of IgG autoantibodies can be detected (3–5, 14, 25, 26). B cell activation in B6.Nba2 was not as great as in NZB mice, and other loci (in addition to Nba2) are likely to contribute to this trait in the NZB strain. Despite the B cell hyperreactivity in both B6.Nba2 and NZB, only the NZB strain manifested expansions of MZ B and B1a cells. In fact, the percentage of MZ B cells was even lower in B6.Nba2 congenics compared with B6 mice. Additional studies indicated that the Nba2 locus is not responsible for expansions of these B cell subsets in (NZB × NZW)F1 mice. Together, the present studies cast doubt on the importance of these B cell subsets in lupus-like disease and provide new insight into how Nba2 contributes to disease in these animal systems.

The present studies show that the Nba2 locus leads to B cell hyperactivity early in life, consistent with the hypothesis that the major lupus-susceptibility gene encoded in this locus is expressed in B cells. Our previous studies (26) suggested that B6.Nba2 mice, like NZB mice, have intrinsic B cell abnormalities, although analyses of mixed chimeras to formally demonstrate this point are still in progress. Experiments also showed that B6.Nba2 mice, compared with B6 mice, greatly overexpress an IFN-inducible gene, Ifi202, which is the major candidate lupus-susceptibility gene within the Nba2 genetic interval (26). Increased expression of this transcriptional regulator was apparent in purified B cells and was associated with apoptotic defects in B cells (26). It remains unclear whether the increased B cell activation is a consequence of the Nba2-encoded defect in B cell apoptosis. However, polyclonal B cell activation early in life is a consequence of certain single gene defects known to interfere with apoptosis of B cells, including...
increased B cell expression of Bcl-2 in transgenic mice (31, 32) and mutation of the gene encoding Fas in lpr mice (32–34).

Several studies have proposed a possible role for MZ B cells in the development of lupus in mouse models. Most relevant to the current studies is the reported expansion of this subset in NZB and (NZB × NZW)F1 mice (4–6). It has been shown that the percentage of the MZ B cell population is significantly increased in (NZB × NZW)F1 as early as 4 wk (5, 16, 35). It was also claimed, based on mapping studies, that the NZB-dependent MZ expansion is linked to Nb2 on distal chromosome 1 and therefore relevant to the development of nephritis (5). Our current studies with Nba2-congenic mice show that this is not correct. CD1<sup>high</sup> B cells in (NZB × NZW)F1 mice have also been shown to produce large amounts of IgM anti-DNA Abs in vitro (19). B6.Nba2 mice and (B6.Nba2 × NZW)F1 mice produce high quantities of IgG anti-nuclear Abs, including IgG Abs to chromatin, dsDNA, ssDNA, and histones (26). The decrease in MZ B cells throughout the course of B6.Nba2 disease cells into question the connection between this subset’s capability to secrete IgM autoantibodies and the production of pathogenic IgG autoantibodies in the course of (NZB × NZW)F1 lupus (3, 5). It has also been shown that mice overexpressing B lymphocyte stimulator (BLYS; also known as B cell-activating factor (BAFF), TNF and apoptosis leukocyte-expressing ligand-1 (TALL-1), and zTNF4) produce autoantibodies and develop a lupus-like disease in association with a marked increase in transitional and MZ B cells (36). Although these and other studies in mice transgenic for anti-DNA Ig genes have shown that autoreactive B cells can accumulate in the marginal zone under various experimental situations (20, 21, 37), it remains to be established whether MZ B cells secrete pathogenic autoantibodies in any model system of lupus.

The absence of MZ B cell expansions in B6.Nba2 mice is consistent with studies that have examined MZ B cells in other lupus models in which disease develops spontaneously. For example, splenic MZ B cells are not expanded in MRL, MRL-Fas<sup>−/−</sup>, or BXSB mice (3, 22). The accelerated development of lupus-like disease in male BXSB mice is due to the presence of an as yet unidentified mutant gene located on the Y chromosome, Y-linked autoimmune acceleration (designated Yaa) (22, 38). The Yaa defect is expressed in B cells (39, 40), and like Nba2, it is associated with B cell hyperactivity, as judged by increased spontaneous IgM secretion (22, 38). Recent studies demonstrated that Yaa mice also have impaired development of MZ B cells, resulting from an effect intrinsic to B cells expressing the Yaa mutation (22). The decreased levels of MZ B cells in both Nba2 and Yaa models of disease argues against a major role of the MZ subset in the generation of pathogenic Abs in lupus.

The mechanisms regulating the differentiation and localization of MZ B cells have not been fully defined. Sensitivity of BCR signaling and factors affecting migration/retention to the MZ have been shown to impact MZ development (22, 29, 41–43). For example, recent studies have demonstrated that interaction of the integrins, LFA-1 and α<sub>4</sub>β<sub>1</sub>, on MZ B cells with their respective ligands, ICAM-1 and VCAM-1, expressed on resident stromal cells in the MZ is critical for the localization and retention of MZ B cells (30). In studies of Yaa mice, the association of B cell hyperactivity and decreased MZ cells supported a model in which increased BCR signaling leads to accelerated maturation of immature transitional B cells toward follicular B cells and therefore away from MZ B cells (22, 41–43). Expression of LFA-1 and α<sub>4</sub>β<sub>1</sub> on MZ and follicular B cells in Yaa mice was not different from those in non-Yaa mice (22), whereas B cell expression of β<sub>1</sub> integrin was significantly reduced in B6.Nba2 compared with B6 mice. These results suggest that the mechanism for defective MZ B cell development in B6.Nba2 mice may be different compared with Yaa mice. In addition, we noted that expression of β<sub>1</sub> integrin was increased on MZ cells in NZB mice, which provides new insight into why these mice have an expanded MZ B cell population.

The importance of B1 cells in the pathogenesis of lupus continues to be debated. A major argument for a role of B1a cells in mouse lupus relates to the expansion of this subset in NZB and (NZB × NZW)F1 mice (7–10). In addition, it is well known that the B1 subset is enriched for cells that produce IgM autoantibodies, particularly natural autoantibodies (41, 44, 45). Natural autoantibodies tend to be polyreactive with low-affinity cross-reactivity to a variety of self Ags (45, 46). These characteristics are very different from the pathogenic IgG Abs produced by lupus mice.

**FIGURE 7.** Percentages of splenic CD<sup>5<sup>-</sup></sup> CD23<sup>−/low</sup>, MZ B, and B1a B cells in the B220 population of (B6 × NZW)F<sub>1</sub>, (B6.Nba2 × NZW)F<sub>1</sub>, (NZB × NZW)F<sub>1</sub>, and parental NZW mice. Data are shown for both 8-wk-old (closed symbols) and 6- to 12-mo-old (open symbols) mice. A. The percentages of CD5<sup>+</sup> CD23<sup>−/low</sup> cells in the splenic B220<sup>−</sup> population are shown for individual mice of each strain. B. The percentages of MZ (CD21<sup>high</sup>CD23<sup>−/low</sup>) cells in the splenic B220 population are shown for individual mice of each strain. C. The percentages of B1a (CD5<sup>+</sup>CD23<sup>−/low</sup>) in the splenic B220 population are shown for individual mice of each strain. D. The percentages of B1a (CD5<sup>+</sup>Mac-1<sup>+</sup>) B cells in the peritoneal B220 population are shown for individual mice of each strain. Horizontal bars indicate the means for each strain. For each analysis, no statistical difference was noted between (B6.Nba2 × NZW)F<sub>1</sub> and (B6 × NZW)F<sub>1</sub>.
Nevertheless, a variety of studies have also shown that manipulations leading to the reduction of B1 cells are associated with amelioration of IgG autoantibody production and disease. For example, lysis of B1 cells via intraperitoneal injection of H2O delayed disease onset and reduced disease severity in (NZB × NZWF1)F1 mice (47). In addition, NZB and (NZB × NZWF1)F1 mice with genetically determined deficiencies of B1 cells, such as in Bruton’s tyrosine kinase (Btk) deficient mice, show reduced development of disease (48). Finally, studies have shown that B1a cells from (NZB × NZWF1)F1 mice are hypersensitive to stromal cell-derived factor 1/CXC chemokine ligand 12, a chemokine that promotes the migration, proliferation, and survival of B1a cells (10, 49). This greater sensitivity was secondary to a NZB genetic effect, and anti-stromal cell-derived factor 1 mAb treatment prevented autoantibody production and nephritis in (NZB × NZWF1)F1 mice in association with inhibition of the B1a cell expansion (10). The interpretation of these different studies in terms of the critical role of B1 cells is made difficult by the fact that none of these manipulations is specific for B1 cells.

Our studies show that a NZB-determined B1a cell expansion is not required for the production of IgG autoantibodies or development of disease in NZB and (NZB × NZWF1)F1 mice. Expansion of B1a cells, like MZ B cells, is therefore determined by a genetic contribution separate from Nba2. Studies in other mouse models of lupus have also suggested that B1a cells are not critical in lupus pathogenesis. For example, B1 cells are not expanded in lupus-prone MLR-Fas(+/−) or BXSX mice (3). Furthermore, reconstitution studies with bone marrow and peritoneal cells have shown that IgG autoantibodies in MRL-Fas(+/−) mice are produced by the conventional (B2) B cell bone marrow source (50).

Most studies of (NZB × NZWF1)F1 mice have focused on B cell abnormalities that derive from the NZB genetic contribution. These include multiple abnormal traits of B lineage precursor cells, B cell hyperactivity and polyclonal B cell activation, hypersensitivity to various activating stimuli, defects in signaling, defects in B cell hyperreactivity present in NZB mice. In conjunction with studies in other lupus-prone strains, the current work adds additional support for models in which the MZ B and B1a cells do not contribute to lupus-like disease, separating their ability to make IgM autoantibodies from the IgG autoantibodies that cause disease.

References


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