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NZB Mice Exhibit a Primary T Cell Defect in Fetal Thymic Organ Culture

Yoshiko Hashimoto,*,† Kenneth Dorshkind, † Encarnacion Montecino-Rodriguez, † Nobuhisa Taguchi,*,† Leonard Shultz,‡ and M. Eric Gershwin 2 *

Defects in T cell development have been suggested to be a factor in the development of systemic autoimmunity in NZB mice. However, the suggestion of a primary T cell defect has often been by extrapolation, and few direct observations of T cell precursors in NZB mice have been performed. Moreover, the capacity of NZB bone marrow T cell precursors to colonize the thymus and the ability of the NZB thymic microenvironment to support T lymphopoiesis have not been analyzed. To address this important issue, we employed the fetal thymic organ culture system to examine NZB T cell development. Our data demonstrated that NZB bone marrow cells were less efficient at colonizing fetal thymic lobes than those of control BALB/c or C57BL/6 mice. In addition, NZB bone marrow cells did not differentiate into mature T cells as efficiently as bone marrow cells from BALB/c or C57BL/6 mice. Further analysis revealed that this defect resulted from an intrinsic deficiency in the NZB Lin “Sca-1+c-kit+” bone marrow stem cell pool to differentiate into T cells in fetal thymic organ culture. Taken together, the data document heretofore unappreciated deficiencies in T cell development that may contribute to the development of the autoimmune phenotype in NZB mice. The Journal of Immunology, 2000, 164: 1569–1575.

The NZB mouse is a well-described model for autoimmune disease, and reports documenting abnormalities of the NZB immune system are extensive (1). In particular, numerous studies have described mature B and T cell defects that include B cell hyperactivity, abnormal T cell function, and production of autoantibodies in NZB mice (2–7). Whether or not defects in primary lymphopoiesis underlie these immune defects has been a topic of considerable interest, and numerous reports have documented abnormalities of primary B lymphopoiesis in these mice (8–13). Clearly, the numbers of B lineage cells in the bone marrow of very young NZB mice exceed control values and then decline rapidly as the mice age. By the time NZB mice are young adults, their bone marrow is markedly deficient in B lineage cells (10–13). More recent studies have demonstrated that this observation correlates with a block at the early pre-B cell stage of development (13). Whether or not defects exist in the bone marrow T cell precursor pool, that would in turn compromise T cell development in NZB mice, has not been investigated.

Thymopoiesis is thought to be dependent upon continuous migration of bone marrow-derived T cell precursors to the thymus (14, 15). Defects in the number of these cells and/or their developmental potential could in turn compromise the production of T lineage cells. Upon entry into the thymus, these precursors interact with the thymic microenvironment and differentiate into mature T lymphocytes that recognize foreign but not self-Ags (16, 17). Abnormalities in the ability of the thymic stroma to support thymopoiesis could also contribute to abnormal T cell development.

The aim of the present study was to assess the ability of NZB bone marrow T cell precursors to colonize the thymus and to analyze the capacity of the NZB thymic microenvironment to support thymopoiesis. These analyses were performed using the fetal thymic organ culture (FTOC) system which permits an evaluation of both these activities (18). The results of these studies indicated that bone marrow T cell precursors in NZB mice do not differentiate in FTOC as efficiently as bone marrow cells from normal mice. In addition, the data also demonstrate an impaired ability of the NZB thymic microenvironment to support T cell maturation.

Materials and Methods

Mice

C57BL/6d (C57BL/6, H-2d, Thy-1.2), NZB/BINJ (NZB; H-2b, Thy-1.2), and BALB/c By (BALB/c; H-2b, Thy-1.2) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were housed in the Division of Laboratory Animal Medicine Vivarium at the University of California, Los Angeles. Timed pregnant Swiss Webster (S/W; H-2k, Thy-1.1) and BALB/c mice were purchased from Taconic Farms (Germantown, NY). Timed pregnant NZB mice were generated in the Animal Resource Services Facility at the University of California, Davis. The presence of a vaginal plug was designated as day 0 of gestation.

Preparation of cell suspensions

Bone marrow cell suspensions were prepared by flushing femurs and tibiae with 3 ml of RPMI 1640. Thymocyte suspensions were prepared by gently pressing thymuses through a fine mesh screen into RPMI 1640. Cells were counted with a hemocytometer and cell viability, determined by eosin dye exclusion, was always >95%.

FTOCs

FTOCs were initiated and maintained based on the protocol established by Jenkinson et al. (19). Briefly, embryonic fetal thymic lobes were aseptically seeded into organ culture dishes containing RPMI 1640 supplemented with 18 U.S.C. Section 1734 solely to indicate this fact.

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Abbreviations used in this paper: FTOC, fetal thymic organ culture; S/W, Swiss Webster; dGuo, deoxyguanosine.

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harvested from day 15 S/W, BALB/c, or NZB embryos. The thymic lobes were dissected free from extraneous tissue and cultured for 5 days in the presence of 1.35 mM deoxyguanosine (dGuo; Sigma, St. Louis, MO) to deplete endogenous lymphohematopoietic cells. The lobes were then rinsed and incubated with 2.5 × 10^6 bone marrow cells in hanging drop cultures in Terasaki plates for 48 h. Subsequently, the lobes were transferred to FTOC on filter/gelfoam rafts in RPMI 1640 supplemented with 10% FCS, 5 × 10^{-5} M 2B-5ME, 100 U/ml streptomycin, and 100 U/ml penicillin and placed in a humidified 37°C in 5% CO_2/air incubator. After 14–21 days of culture, thymocytes were harvested by gently pressing the lobes through fine mesh sieves with the plunger of a sterile 1-ml syringe. Donor cell origin was confirmed by immunofluorescence on the basis of Thy-1 allotypic differences. During each FTOC experiment, 3–10 pregnant mice were sacrificed, and 8–40 lobes were used for organ culture. Bone marrow cells pooled from at least two mice per strain were used as donor cells. It is necessary to analyze thymus repopulation per condition on pooled lobes to obtain enough cells for analysis; too few cells per lobe can be harvested to do analyses on a per lobe basis. Within any single FTOC assay, results are based on pooled data from several pregnant mice and embryos. There is an inherent variability in the FTOC assay and hence experiments are presented independently. However, all data between experiments are consistent as noted below.

Flow cytometry
Cells were analyzed for the expression of the following cell surface determinants: Thy-1.2 (clone 53-2.1), CD4 (clone RM4-5), CD8 (clone 53-6.7), CD5 (clone 53-7.3), IgM (clone 53-2.1), and/or CD45R (B220, clone RA3-6B2) using Abs conjugated to fluorescein, PE, or biotin (PharMingen, San Diego, CA). Biotinylated Abs were revealed with PerCP-streptavidin (Becton Dickinson, San Jose, CA). The optimal working dilution was determined for each Ab before use. Before staining, FcγRIII receptors on cells were blocked by preincubation of the samples with a rat anti-CDw32/16 Ab (PharMingen). Cells were then incubated with the first step Ab for 30 min at 4°C, washed with Ca^{2+}- and Mg^{2+}-free PBS, and incubated for an additional 30 min at 4°C with the second step reagent. After the last wash, the cells were resuspended in Ca^{2+}- and Mg^{2+}-free PBS, and viable cells from each sample were analyzed on a Becton Dickinson FACScan (Becton Dickinson).

Isolation and sorting of CD45R<sup>-</sup> bone marrow cells
Bone marrow cells from 4- to 8-wk-old mice were labeled with FITC-conjugated anti-CD45R Ab (PharMingen), and CD45R<sup>-</sup> cells were isolated using a Becton Dickinson FACStar. Reanalysis showed that purity of the sorted population was >99%. The capacity of sorted CD45R<sup>-</sup> cells to repopulate fetal thymic lobes was determined by incubating 1 × 10^6 cells with dGuo-treated lobes as described above.

Isolation and sorting of lineage marker-negative (Lin<sup>-</sup> Sca-1<sup>+</sup> c-kit<sup>-</sup>) bone marrow cells
Lineage depleted (Lin<sup>-</sup>) bone marrow cells were isolated from femurs and tibiae of five to seven BALB/c, C57BL/6, or NZB mice as described previously (20, 21). Briefly, bone marrow cells were incubated in PBS at 4°C for 30 min in a mixture of optimal concentrations of rat Abs against the lineage-specific Ags CD2 (clone RM2-5), CD8 (clone 53-6.7), CD45R (clone RA3-6B2), Gr-1 (clone RB6-8C5), Mac-1 (clone M1/70), and TER-119 (PharMingen). The washed cells were then exposed to anti-rat IgG-conjugated MicroBeads (Miltenyi Biotec, Germany) for 30 min at 4°C and subsequently passed through a magnetic field using a magnetic cell separation system separation column. Cells binding these Abs were retained on the column while the Lin<sup>-</sup> cells were recovered in the eluate. This Lin<sup>-</sup> fraction was further purified by sorting on a FACStarplus flow cytometer (Becton Dickinson) following a 20-min incubation with FITC-conjugated anti-rat IgG to exclude Lin<sup>-</sup>-contaminating cells. Reanalysis showed that the purity of the sorted Lin<sup>-</sup> cells obtained was >97%. Sorted Lin<sup>-</sup> bone marrow cells (500–10,000) were then incubated with dGuo-treated thymic lobes in FTOC as described above.

In some experiments, the Lin<sup>-</sup> fraction obtained following magnetic depletion was incubated with a Tri-Color-conjugated anti-rat IgG for 20 min. After washing, Tri-Color-anti-rat IgG-free binding sites were blocked by incubation with normal rat IgG (1 μg/10^5 cells) before the addition of PE-anti-c-kit (clone 2B8) and FITC-conjugated anti-Sca-1 (Ly6A/E, clone E13-161.7). Then, Lin<sup>-</sup> cells expressing Sca-1 and c-kit were purified using a FACStarplus (see Refs. 22 and 23; Becton Dickinson) and graded doses (50–500) of Lin<sup>-</sup> Sca-1<sup>+</sup> c-kit<sup>-</sup> cells were then incubated with dGuo-treated thymic lobes in FTOC as described above.

**Statistics**
A two-tailed Student’s t test was used to determine the significance of the data.

**Results**
**Thymus-repopulating potential of NZB bone marrow cells**
Bone marrow progenitor cell abnormalities have been described in lupus-prone mice (12, 13, 24), but the direct thymus-repopulating capacity of NZB bone marrow cells has not been examined. To assess their T cell developmental potential, bone marrow cells from age-matched NZB and C57BL/6 mice were used to reconstitute dGuo-treated fetal thymic lobes. The thymic lobes used in each individual experiment were obtained from the same pool of
embryos. After 14 days of culture, thymocytes were harvested and a donor T cell development, based on expression of the Thy-1.2 allele, was measured.

As shown in Fig. 1A, the number of cells recovered from thymic lobes repopulated with bone marrow cells from 1-mo-old C57BL/6 or NZB mice was comparable. However, although the total number of thymocytes recovered varied from experiment to experiment, within a particular experiment, bone marrow cells from NZB mice 2 mo of age and older were not as efficient at repopulating thymic lobes as age-matched cells from C57BL/6 mice (p < 0.02). In addition, bone marrow cells from NZB mice of all ages analyzed showed a reduced capacity to differentiate into CD4+ and CD8+ cells (p < 0.01) cells (Fig. 1B). On the other hand, while the frequencies of CD4+ and CD8+ cells were slightly reduced in lobes repopulated with NZB bone marrow, these differences were not significant (Fig. 1B).

Reduced thymic repopulating potential of NZB mice is not due to MHC mismatch

S/W mice were used in these studies because their large litter size made it possible to harvest a large number of thymic lobes from a single pregnant female. Furthermore, the Thy-1.1 allotype of the S/W strain made it possible to distinguish donor Thy-1.2 cells from endogenous thymocytes. However, a consequence of using the outbred S/W strain is that the NZB bone marrow cells used for reconstitution differentiate in a nonhistocompatible thymic microenvironment. NZB mice express the H-2d haplotype whereas S/W mice express the recombinant H-2 s/q haplotype. Complicating matters further is that there is no ideal control strain to which NZB mice can be compared. In view of these points, experiments were performed to determine whether the above results were due to histocompatibility differences.

In experiments 1 and 2 in Table I, bone marrow cells from 3-mo-old NZB (H-2d), BALB/c (H-2d), and C57BL/6 (H-2b) mice were used to repopulate S/W thymic lobes. The use of BALB/c bone marrow cells allowed the T cell precursor potential of another source of H-2d cells to be evaluated in the S/W H-2 s/q environment. Results from this experiment demonstrated that NZB marrow cells were less efficient than cells derived from either C57BL/6 or BALB/c mice in repopulating thymic lobes. Both the total number of cells recovered per lobe and the frequency of CD4+ and CD8+ cells was lower in lobes repopulated with NZB bone marrow cells. In experiment 3, BALB/c, C57BL/6, and NZB bone marrow cells were used to repopulate thymic lobes from BALB/c mice. In this

### Table I. Defective thymus repopulating potential of NZB bone marrow cells in FTOC is not due to MHC differences

<table>
<thead>
<tr>
<th>Expt</th>
<th>Donor (MHC haplotype)</th>
<th>Recipient (MHC haplotype)</th>
<th>Donor Cell No./Lobe a</th>
<th>% Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(×10^4)</td>
<td>CD4−CD8+</td>
</tr>
<tr>
<td>1</td>
<td>BALB/c (d)</td>
<td>S/W (outbred)</td>
<td>8.8</td>
<td>77.8</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 (b)</td>
<td></td>
<td>6.7</td>
<td>75.5</td>
</tr>
<tr>
<td></td>
<td>NZB (d)</td>
<td></td>
<td>5.2</td>
<td>85.4</td>
</tr>
<tr>
<td>2</td>
<td>BALB/c (d)</td>
<td>S/W (outbred)</td>
<td>7.6</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 (b)</td>
<td></td>
<td>8.7</td>
<td>79.0</td>
</tr>
<tr>
<td></td>
<td>NZB (d)</td>
<td></td>
<td>6.1</td>
<td>86.4</td>
</tr>
<tr>
<td>3</td>
<td>BALB/c (d)</td>
<td>BALB/c (d)</td>
<td>7.4</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 (b)</td>
<td></td>
<td>8.2</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td>NZB (d)</td>
<td></td>
<td>4.7</td>
<td>90.4</td>
</tr>
</tbody>
</table>

* Bone marrow cells from 3-mo-old mice were used as donor cells. Data were calculated based on analysis of at least 15 lobes/experiment.

a Differences in cell recovery between lobes repopulated with either BALB/c or C57BL/6 and NZB bone marrow cells are significant at p < 0.1 and p < 0.05, respectively.

b Differences in the frequency of CD4+ CD8+ cells between lobes repopulated with BALB/c and NZB bone marrow cells are significant at p < 0.05.

c Differences in the frequency of CD4−CD8+ cells between S/W lobes repopulated with either BALB/c or C57BL/6 and NZB Lin− cells are significant at p < 0.02.

d In Expt. 1, all mice were 2 mo old.

e In Expt. 2, all mice were 4 mo old.

### Table II. Colonization of fetal thymic lobes using limiting numbers of Lin− bone marrow cells from BALB/c, C57BL/6, and NZB mice

<table>
<thead>
<tr>
<th>Expt</th>
<th>Strain</th>
<th>No. of Donor Cells</th>
<th>Donor Cell No./Lobe a</th>
<th>% Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(×10^4)</td>
<td>CD4−CD8+</td>
</tr>
<tr>
<td>1</td>
<td>BALB/c</td>
<td>1,000</td>
<td>25.3</td>
<td>54.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,000</td>
<td>39.7</td>
<td>38.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10,000</td>
<td>46.0</td>
<td>39.2</td>
</tr>
<tr>
<td></td>
<td>NZB</td>
<td>1,000</td>
<td>14.2</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,000</td>
<td>26.0</td>
<td>70.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10,000</td>
<td>33.0</td>
<td>70.0</td>
</tr>
<tr>
<td>2</td>
<td>C57BL/6</td>
<td>500</td>
<td>5.1</td>
<td>55.3</td>
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<td></td>
<td></td>
<td>1,000</td>
<td>8.8</td>
<td>42.2</td>
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<td></td>
<td></td>
<td>2,000</td>
<td>11.6</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>NZB</td>
<td>500</td>
<td>3.9</td>
<td>80.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,000</td>
<td>5.3</td>
<td>77.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,000</td>
<td>8.4</td>
<td>78.6</td>
</tr>
</tbody>
</table>

a FTOC assayed at 3 wk. Data were calculated based on analysis of at least 15 lobes/experiment.

b Differences in cell recovery between S/W lobes repopulated with either BALB/c or C57BL/6 and NZB Lin− cells are significant at p < 0.005 and p < 0.1, respectively.

c Differences in the frequency of CD4−CD8+ cells between S/W lobes repopulated with either BALB/c or C57BL/6 and NZB Lin− cells are significant at p < 0.01 and p < 0.05, respectively.
case, the NZB and BALB/c bone marrow cells were seeded in a MHC-matched thymic microenvironment. As observed in the previous experiments, NZB bone marrow cells had a reduced capacity to repopulate fetal thymic lobes when compared with BALB/c and C57BL/6 bone marrow cells. In addition, NZB bone marrow cells did not differentiate into CD4⁺CD8⁻ cells to the same level as bone marrow cells from the other two nonautoimmune strains. Therefore, these results strongly suggest that the inefficiency of NZB bone marrow cells to repopulate the thymus is not an artifact that results from MHC differences.

Intrinsic stem cell defects are demonstrable in NZB mice

Taken together, the above results suggested that bone marrow cells from NZB mice do not efficiently repopulate the thymus. One explanation for this observation is that intrinsic defects in stem cells or more committed T cell progenitors exist.

To investigate this possibility, Lin⁺ bone marrow cells from BALB/c, C57BL/6, or NZB mice were isolated, and limiting numbers of cells were incubated with S/W thymic lobes in FTOC. Because the Lin⁺ population is enriched in very early precursor populations (20, 21), the thymic lobes were incubated with donor cells for 3 wk instead of the 2-wk period used with unseparated bone marrow cells. As indicated in Table II, at each cell dose tested, the total number of donor cells recovered and the frequency of CD4⁺CD8⁻ cells was lower in the lobes repopulated with NZB Lin⁻ cells.

To determine whether this defect could be traced to more primitive progenitors in the bone marrow, the thymic repopulating potential of Lin⁻ Sca-1⁺ c-kit⁺ cells, which are highly enriched in stem cell activity (22, 23) from BALB/c, C57BL/6, or NZB mice, was compared. Again, as previously observed with total bone marrow and Lin⁻ cells, NZB Lin⁻ Sca-1⁺ c-kit⁺ cells showed a reduced ability to reconstitute S/W lobes (Fig. 2 and Table III).

Table III. Colonization of fetal thymic lobes using limiting numbers of Lin⁻ Sca-1⁺ c-kit⁺ bone marrow cells from BALB/c, C57BL/6, and NZB mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of Donor Lin⁻ Sca-1⁺ c-kit⁺ cells</th>
<th>CD4⁺CD8⁻</th>
<th>CD4⁺CD8⁻</th>
<th>CD4⁺CD8⁺</th>
<th>CD4⁺CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>50</td>
<td>80.7</td>
<td>7.0</td>
<td>3.0</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>77.3</td>
<td>11.0</td>
<td>4.0</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>56.9</td>
<td>18.9</td>
<td>4.3</td>
<td>19.9</td>
</tr>
<tr>
<td>C57/BL6</td>
<td>50</td>
<td>59.9</td>
<td>17.7</td>
<td>5.0</td>
<td>18.1</td>
</tr>
<tr>
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<td>200</td>
<td>59.8</td>
<td>20.3</td>
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<td>41.8</td>
<td>27.1</td>
<td>5.5</td>
<td>25.6</td>
</tr>
<tr>
<td>NZB</td>
<td>50</td>
<td>86.9</td>
<td>2.4</td>
<td>1.1</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>79.4</td>
<td>5.1</td>
<td>0.8</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>70.0</td>
<td>11.1</td>
<td>4.7</td>
<td>14.1</td>
</tr>
</tbody>
</table>

a FTOC assayed at 3 wk. Data were calculated based on analysis of at least eight lobes per experiment. Two-month-old mice were used as donors.

b Differences in the frequency of CD4⁺CD8⁻ between S/W lobes repopulated with either BALB/c or C57BL6 and NZB Lin⁻ Sca-1⁺ c-kit⁺ cells are significant at p < 0.01 and p < 0.001, respectively.

Capacity of NZB thymic microenvironment to support thymopoiesis

To assess the ability of the NZB thymic microenvironment to support T cell development, thymic lobes from day 15 NZB embryos were seeded with bone marrow cells from NZB or C57BL/6 mice. Because all mice express the Thy-1.2 allele, it was not possible to use differences in Thy-1 expression to confirm the donor origin of cells. However, fewer than 10³ cells were ever harvested from dGuo-treated NZB lobes cultured without exogenous cells (data not shown). Thus, reconstitution by endogenous precursors was deemed to be negligible.

As shown in Fig. 3, NZB fetal thymic lobes were able to support the differentiation of bone marrow cells from C57BL/6 mice. Moreover, as previously seen with S/W lobes, fewer cells were recovered from lobes seeded with NZB bone marrow cells 2 mo of age and older (Fig. 3A). Surprisingly, however, a deficiency in the number of cells harvested from lobes seeded with 1-mo-old donors was also observed. In addition, as with S/W lobes, NZB bone marrow cells, regardless of age, exhibited an impaired capacity to differentiate into CD4⁺CD8⁻ cells (p < 0.05) as compared with donor cells from C57BL/6 mice (Fig. 3B).
Discusson

The thymus is the site of primary T cell development (25–27), and thymopoiesis is thought to be sustained by the continuous migration of bone marrow-derived T cell precursors (14). These cells then interact with a complex microenvironment composed of epithelial, fibroblastoid, and hematopoietically derived stromal cell populations. Because abnormal T cell development could result in the generation of autoreactive T cells, there has been considerable interest in dissecting the process of thymopoiesis in NZB mice. In view of the well-defined, premature depletion of bone marrow B cell progenitors in NZB mice, whether or not a similar accelerated decline in the bone marrow T cell precursor pool occurs in that strain was of particular interest (10–13). Accordingly, the aims of the present study were to examine the thymic repopulating potential of NZB bone marrow cells and to investigate the capacity of the NZB thymic microenvironment to support T cell development.

Initial studies compared the T cell repopulating potential of NZB bone marrow to that of normal mice. These studies demonstrated a deficiency in the thymus-repopulating capacity of NZB bone marrow from mice 2 mo of age and older. Both the overall cellularity and the frequency of differentiated thymocytes were lower in fetal thymic lobes repopulated with NZB bone marrow than in lobes repopulated with age-matched cells from normal mice. This result was not an experimental artifact resulting from histocompatibility differences between the repopulating bone marrow cells and the thymic microenvironment. When the ability of NZB and BALB/c bone marrow cells, which both share the H-2d haplotype, to repopulate the S/W thymus was compared, thymopoiesis was defective only in the lobes repopulated with the NZB cells. That bone marrow cells from 2-mo-old NZB mice had a reduced thymus-repopulating potential was surprising, because defects in the bone marrow T cell precursor pool are usually not observed in mice of that age (28–30). One possible explanation for this observation is the existence of intrinsic defects in NZB stem cells and/or committed T cell precursors.

To examine this possibility, the thymus-repopulating potential of comparable numbers of Lin− bone marrow cells from NZB, BALB/c, and C57BL/6 mice was compared under limiting dilution conditions. The results clearly demonstrated that NZB cells were not as efficient at repopulating the thymus or in differentiating into CD4+ CD8+ cells as were C57BL/6 Lin− cells. Further analysis with Lin− Sca-1− cKt− cells, a population enriched for stem cell activity, corroborated these findings. At all cell doses examined, the fetal thymic lobes repopulated with NZB cells contained fewer cells and a lower frequency of CD4+ CD8+ thymocytes. Taken together with our preliminary data showing CD4− CD8− NZB thymocytes proliferate as well as DN cells from BALB/c mice in response to IL-7, these results indicate that intrinsic defects in the stem cell/prothymocyte pool exist in young NZB mice before the onset of autoimmune disease. Further work is needed, as noted below, to address this thesis.

FIGURE 3. Thymic lobes from day 15 NZB embryos were reconstituted with C57BL/6 or NZB marrow cells. A, Cell number per lobe recovered after 14 days in FTOC with the indicated bone marrow cell source. B, Representative FACScan profile for thymocytes from reconstituted fetal thymic lobes. The percentage of cells expressing indicated markers is shown in each quadrant. The data shown are representative of five experiments. Cells from at least 15 lobes were pooled for analysis in each experiment. The number of cells recovered from S/W lobes repopulated with NZB bone marrow cells is reduced, but these differences are not significant. Differences in the frequency of CD4+ CD8+ cells between lobes repopulated with C57BL/6 and NZB bone marrow cells are significant at p < 0.05. Values are evaluated by Student’s t test based on five independent experiments.

FIGURE 4. Intrathymic B cell development in NZB lobes reconstituted with C57BL/6 or NZB marrow cells. A, Frequency of Thy-1+ cells in the reconstituted lobes. B, Frequency of CD45R+ and surface IgM+ in thymocytes harvested from fetal thymic lobes. Note that the frequency of Thy-1+ expressing T cells in the NZB thymus is lower than in the C57BL/6 thymus due to the increased number of NZB B lineage cells and that the frequency of (CD45R+) B lineage cells combined with that of the Thy-1+ cells equals 100%. Cells from at least 15 lobes were pooled for analysis in each experiment.
The capacity of the NZB thymic microenvironment to support T cell development was also examined in this study by seeding NZB fetal thymic lobes with NZB and C57BL/6 bone marrow cells. C57BL/6 bone marrow cells differentiated normally into T cells in the NZB thymus. This result was not unexpected, since NZB mice are not deficient in T cells. Furthermore, comparable to what was observed with S/W thymic lobes, a defect in the capacity of donor NZB bone marrow cells to differentiate into CD4^+ CD8^- thymocytes was observed. However, surprisingly, the cellularity in NZB thymic lobes repopulated with NZB bone marrow cells from 1-mo-old mice was lower than in lobes repopulated with age-matched C57BL/6 bone marrow cells. Such a deficiency was not observed when cells from 1-mo-old NZB mice were used to repopulate S/W thymic lobes. One explanation for this latter result could be that defects in the NZB thymic microenvironment further compromise deficiencies in the bone marrow T cell precursor pool in that strain.

Further evidence that the NZB thymic microenvironment differs from that in other strains of mice was that an anomalously high level of B lymphopoiesis occurred in NZB fetal thymic lobes repopulated with NZB bone marrow cells. Interestingly, although the thymus is known to contain a minor subpopulation of B lymphocytes (31–34), this defect was only observed when bone marrow cells from young NZB donors were used to repopulate NZB thymuses. No differences in thymic B cell frequency were demonstrable when NZB donor cells were derived from 6-mo-old mice or when NZB donor cells of any age were seeded into S/W lobes. As previously discussed, B lineage cells in young NZB mice are present at elevated levels and then undergo an accelerated maturation process that results in a B cell precursor deficiency in older animals (10–13). The present results suggest that the same process is paralleled in the thymic microenvironment as well.

Although FTOC is a time-consuming and relatively inefficient in vitro system, it does allow for study of the full T cell development potential of thymic populations. These cultures are the only in vitro system that preserves the three-dimensional organization of the thymus necessary for the full process of differentiation. More important, it allows manipulation of embryonic rudiments to produce chimeric thymuses to allow the system to be exploited for the study of stromal lymphoid interactions. Using this system, these studies have revealed that there is a primary defect in T cell development in NZB mice. This finding takes on significance not only because it is such an early event in immune ontogeny, but also because it explains a number of previous observations in NZB T cell biology. For example, it has been known for many years that NZB mice undergo premature thymic involution (35).

The present studies suggest at least two nonmutually exclusive ways in which the stem cell abnormalities described herein may result in abnormal thymopoiesis in NZB mice. The first possibility would be operative at the level of the stem cell/prothymocyte. If stem cells exhibit a defect in their capacity to migrate into the thymus, then fewer numbers of precursors would be present in that organ with a concomitant decline in the number of thymocytes produced. Although our preliminary studies do not reveal a proliferative defect in the immature, CD4^-CD8^- NZB intrathymic progenitor pool (data not shown), additional developmental defects that contribute to the NZB deficit in T cell production may nevertheless be manifest once T cell precursors enter the thymus. A second possibility is that NZB hematopoietic precursors are defective in their ability to generate accessory microenvironmental cell populations required for normal thymopoiesis. It is known that components of the thymic microenvironment are marrow derived and that their presence is necessary for normal thymopoiesis (35–37). If NZB stem cells fail to generate such populations or those that do develop are abnormal, this could affect the integrity of the NZB thymic microenvironment. In this regard, it has been demonstrated using panels of mAbs directed at the thymic microenvironment that NZB mice have thymic stromal cell defects (35). Clearly, modifications of the thymic architecture may be disruptive for both positive and negative selection. The studies reported herein are the first to have assessed the capacity of the NZB thymic microenvironment to support thymopoiesis. Future studies are aimed at investigating these issues and determining whether or not the stem cell defects reported herein contribute to the development of autoimmune disease or represent an independent defect of the NZB strain.

References