Terminal Deoxynucleotidyl Transferase Deficiency Decreases Autoimmune Disease in MRL-Fas<sup>lpr</sup> Mice

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Terminal Deoxynucleotidyl Transferase Deficiency Decreases Autoimmune Disease in MRL-Fas<sup>lpr</sup> Mice<sup>1,2</sup>

Ann J. Feeney,<sup>3</sup> Brian R. Lawson, Dwight H. Kono, and Argyrios N. Theofilopoulos

The neonatal Ab and TCR repertoires are much less diverse, and also very different from, the adult repertoires due to the delayed onset of terminal deoxynucleotidyl transferase (TdT) expression in ontogeny. TdT adds nontemplated N nucleotides to the junctions of Iggs and TCRs, and thus its absence removes one of the major components of junctional diversity in complementarity-determining region 3 (CDR3). We have generated TdT-deficient MRL/lpr, Fas-deficient (MRL–Fas<sup>lpr</sup>) mice, and show that they have an increased lifespan, decreased incidence of skin lesions, and much lower serum levels of anti-dsDNA, anti-chromatin, and IgM rheumatoid factors. The generalized hypergammaglobulinemia characteristic of MRL–Fas<sup>lpr</sup> mice is also greatly reduced, as is the percentage of CD4<sup>+</sup>CD8<sup>+</sup>B220<sup>+</sup> (double-negative) T cells. IgG deposits in the kidney are significantly reduced, although evidence of renal disease is present in many mice at 6 mo. CDR3 regions of both IgH and TCR from peripheral lymphocytes of MRL–Fas<sup>lpr</sup> mice are shorter in the absence of TdT, and there is a paucity of arginines in the IgH CDR3 regions of the MRL–Fas<sup>lpr</sup> TdT<sup>−</sup>/− mice. Because the amelioration of symptoms is so widespread, it is likely that the absence of N regions has more of an effect than merely decreasing the precursor frequency of anti-dsDNA B cells. Hence, either the T or B cell repertoires, or more likely both, require N region diversity to produce the full spectrum of autoimmune lupus disease. The Journal of Immunology, 2001, 167: 3486–3493.
very surprising that these (NZB × NZW)F1 TdT−/− mice produced similar levels of anti-dsDNA to their wild-type counterparts. Because TdT is expressed only in developing T and B cells, and its function is to add N region diversity, the decrease in pathology in these mice is likely to be due to a repertoire change in either B or T cells, or both. Thus, these studies either suggested that the limitation in the T or B cell repertoire due to the absence of N region nucleotides was for a different specificity, or that the fine specificity or affinity for dsDNA might be very different in the two groups of mice.

In this study we bred TdT-deficient mice to MRL/lpr, Fas-deficient (MRL-Faslr) mice to determine whether the decrease in autoimmune disease due to TdT deficiency was generalizable to another lupus mouse model. Also, we were interested in examining the anti-dsDNA Abs, as well as to broaden the spectrum of autoimmune disease due to TdT deficiency. Thus, essentially all aspects of the lupus disease were lessened in these mice due to the absence of TdT.

Materials and Methods

Mice

MRL-Faslr mice were obtained from the breeding colony at The Scripps Research Institute. TdT−/− mice, backcrossed approximately three generations to C57BL/6, were given to us by Drs. D. Mathis and C. Benoist (both from Harvard University, Cambridge, MA) via Dr. M. Bevan (University of Washington, Seattle, WA), and have been maintained as a standard Ig-calibrated mouse sera (The Binding Site Limited, Birmingham, AL). Ab concentrations were determined by comparison to standard Ig-calibrated mouse sera (The Binding Site Limited, Birmingham, U.K.).

Serology

 Serum levels of polyclonal IgG, anti-dsDNA, anti-technatin, and IgM-RF were determined by ELISA. Wells were coated with either Fc-specific F(ab′)2 of goat anti-mouse IgG (3 μg/ml; Jackson ImmunoResearch Laboratories, West Grove, PA), mouse chromatin (3.5 μg/ml, a gift from Dr. R. Rubin, The Scripps Research Institute, La Jolla, CA), or, after overnight pre coating with poly-t-lysine, dsDNA (3.5 μg/ml; Sigma). Bound serum total IgG and IgG subclasses were measured using alkaline phosphatase-conjugated goat anti-mouse IgG and subclass-specific Abs (Caltag Laboratories, Burlingame, CA). Serum IgM-RF levels were determined by adding diluted sera to wells coated with purified IgG subclasses (3 μg/ml; all obtained from Caltag Laboratories, except IgG2a, which was purchased from BD PharMingen) and then developing with alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). Ab concentrations were determined by comparison to standard Ig-calibrated mouse sera (The Binding Site Limited, Birmingham, U.K.).

Immunohistology

Detecting Abs included FITC-conjugated anti-mouse IgG (Vector Laboratories, Burlingame, CA), biotinylated anti-mouse CD3 (BD PharMingen), and biotinylated F4/80 (Caltag Laboratories). Standard immunohistologic staining procedures were used. Briefly, OCT-embedded, snap-frozen kidneys were sectioned, air-dried, and acetone-fixed. Nonspecific background was reduced by an avidin/biotin blocking kit (Vector Laboratories) and incubated with 10% goat or rabbit serum in PBS. Sections stained with FITC-conjugated Abs were directly examined, whereas sections stained with biotinylated Abs were incubated with streptavidin-HRP, developed with aminoethyl carbazole (Vector Laboratories), and counterstained with Mayer’s hematoxylin. Sections were graded blindly on a 1–4 scale.

CDR3 analyses

DNA from spleens of 4.5-mo-old N5 mice was prepared as previously described (18). VHJ558 rearrangements were amplified using the Vμ primer AF326 (5′-GGGCGTTAAACGCGTTGTCACCGAAGGCT) with the previously described Jμ primers (Jμ1, 4 and Jμ2, and Jμ3) (19). Amplified products from three independent PCR for each group of mice were cloned and sequenced as previously described (19). RNA was prepared with TRIzol from sorted B220+ T cells from lungs of 5-mo-old N5 mice, and from CD4+ spleen cells from 4-mo-old N5 mice. Rearranged Vβ8 transcripts were amplified with a Vβ8 and a Cβ primer set as previously described (20). Amplified products from three to four independent PCRs were cloned and sequenced from each group of mice.

Statistics

Survival was analyzed by Kaplan-Meier statistic using censored events with significance determined by log-rank (Mantel-Cox) test. The unpaired t-test, Mann-Whitney U test, or Fisher exact test were used to compare groups as indicated. Values of p < 0.05 were considered significant.

Results

Increased survival of TdT-deficient MRL-Faslr background mice

TdT+/− and TdT−/− MRL-Faslr mice were produced by intercrossing TdT+ Fas−/TdT− Faslr heterozygous backcross mice. The first group of intercross mice, consisting of 13 N2 and 25 N3 mice, were analyzed for survival up until 6 mo, when the survivors were sacrificed. Despite the limited backcrossing, the median survival of TdT wild-type mice (5.6 mo) was similar to that of our MRL-Faslr colony (~5.5 mo) (Fig. 1). In contrast, TdT-deficient mice had increased survival with 83.5% (4 of 5 N2 and 10 of 12 N3 mice) alive at 6 mo compared with only 47.6% (4 of 8 N2 and 6 of 13 N3 mice) of TdT+ mice (p = 0.04) (Fig. 1). An additional 10 mice were followed until 7.5 mo of age. At that time, 5 of the TdT− mice were still alive, whereas only 2 of 5 of the TdT+ mice were alive. Thus, TdT deficiency greatly increases the lifespan of MRL-Faslr mice.

Pathology of TdT-deficient mice

One striking macroscopic finding was a marked reduction in the incidence of skin lesions in the TdT-deficient MRL-Faslr mice. Of the N3 mice, only 1 of 9 TdT−/− MRL-Faslr mice had skin....
lesions vs 4/5 of TdT+/+ MRL-Fasbr mice at 6 mo of age, when the mice were sacrificed for histologic examination. An additional 19 N5 intercross mice that were 6 mo old were also examined for skin lesions, and 60% of the TdT+/+ mice but only 22% of the TdT-deficient mice displayed skin lesions (Table I).

Direct immunofluorescence examination of kidney sections of the N3 mice that survived until 6 mo revealed significantly less IgG deposits in the glomeruli of TdT−/− animals (p = 0.0005) (Table I and Fig. 2). Both mesangial and capillary wall deposits were observed in both groups, although the amount of staining was greatly reduced in the TdT−/− animals. Despite the reduction in IgG deposits, no decrease in GN scores was detected in the TdT−/− group (Table I). It should be recalled that half of the TdT−/− mice, but few of the TdT+/+ mice, had already died before this 6-mo time point, and these mice presumably all had severe GN. Thus, although TdT−/− mice have reduced early mortality, a significant number develop some pathologic evidence of glomerular injury by 6 mo of age.

**Lymphocyte subsets and proliferation in TdT-deficient MRL-Fasbr mice**

The point mutation in the Fas gene results in massive lymphocytocaccumulation, and the presence of large numbers of T cells with the unusual phenotype of B220 CD4−CD8− αβTCR+ (DN B220 T cells). MRL-Fasbr mice also develop increases in memory/effector phenotype (CD44high) T cells. The TdT-deficient MRL-Fasbr mice displayed a 2-fold reduction in the percentage of B220+ DN T cells in cervical LN and spleen as compared with TdT wild-type littermates (p < 0.0002) (Fig. 3). As might be expected with the decrease in B220+ DN cells, we also observed a decrease in the lymphadenopathy, which is a hallmark of MRL-Fasbr mice in TdT−/− mice (Table II). B cells, CD8+, and CD4+ cells were compensatorily increased in proportion in spleen and LNs of TdT-deficient MRL-Fasbr mice by 1.5- to 2.5-fold (p = 0.009 and 0.017 for B cells; p = 0.04 and 0.008 for CD8+ cells, p = 0.008 and 0.003 for CD4+ cells). Activated CD4+CD44high and CD8+CD44high T cells were not significantly reduced in either organ, but DN CD44high T cells in both organs were modestly but significantly reduced in the TdT-deficient animals as compared with their TdT+/+ littermates (p = 0.011 and 0.03).

To determine whether the percentage of cycling cells was reduced in the TdT-deficient MRL-Fasbr mice, three TdT+/+ and three TdT−/− 3-mo-old mice from the N4 generation were fed BrdU over a 9-day period. There was no difference in the proportion of BrdU-labeled B220+ DN T cells or B cells in the spleen. However, we did observe a decrease in the percentage of labeled CD4+CD44high and CD8+CD44high T cells, but only the reduction in CD4+ cells achieved statistical significance (p = 0.011 and p = 0.127, respectively) (Fig. 4).

**Serologic analysis of TdT-deficient mice**

As observed in wild-type MRL-Fasbr mice, the N3 generation TdT-expressing MRL-Fasbr mice exhibited marked hypergammaglobulinemia and elevated anti-chromatin and anti-dsDNA responses, and produced significant amounts of IgM-RF (21). In contrast, TdT−/− MRL-Fasbr mice displayed a 57% reduction in total IgG (143 ± 37 vs 62 ± 21 mg/ml) and an even greater reduction of anti-chromatin Abs (151 ± 28 vs 52 ± 24 ng/ml), anti-dsDNA Abs (68 ± 18 vs 24 ± 9 ng/ml), and IgM-RF (1005 ± 241 vs 290 ± 77 ng/ml), compared with their TdT wild-type littermates (Fig. 5). In the case of the polyclonal IgG, the only significant reduction was in the IgG2a subclass (p = 0.04), whereas for the anti-dsDNA autoantibodies, a significant reduction was observed in both IgG2a and IgG2b subclasses (p = 0.02), and a reduction in IgG1 was also seen (p = 0.055). IgM-RF Abs reactive with all IgG subclasses were significantly decreased (p values ranging from 0.05 to 0.0003). Although the reduction in total levels of these specific autoantibodies exceeded the reduction in total polyclonal IgG, most of the autoantibodies and polyclonal Ig are of the IgG2a subclass, and all IgG2a values were reduced ∼3-fold. Thus, the decrease in total IgG2a could account for the decrease in IgG2a autoantibody titers, thus leaving it unclear whether there was a specific decrease in autoantibody levels in addition to the generalized decrease in hypergammaglobulinemia.

**CDR3 regions of IgH rearrangements**

To analyze CDR3 diversity in VDJ rearrangements, we amplified VH558 rearrangements from splenic DNA of 4.5-mo-old littermate MRL-Fasbr TdT−/− or TdT+/+ mice. As expected, the CDR3 regions of Ig from the TdT−/− mice were 1.1 aa shorter than those of the TdT+/+ mice (Table III). Importantly, only one of the 38 IgH rearrangements from the MRL-Fasbr TdT−/− mice had any arginines in CDR3, whereas 10/21 of the CDR3 regions

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**Table I. Pathology results for TdT−/− and TdT+/+ MRL-Fasbr mice**

<table>
<thead>
<tr>
<th>Glomerular IgG Deposits</th>
<th>GN Score</th>
<th>Skin Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdT+/+</td>
<td>3.25 ± 0.4</td>
<td>2.0 ± 0.36</td>
</tr>
<tr>
<td>TdT−/−</td>
<td>1.3 ± 0.2</td>
<td>2.6 ± 0.22</td>
</tr>
<tr>
<td>p values</td>
<td>0.0005</td>
<td>0.043</td>
</tr>
</tbody>
</table>

* Mean ± SEM for all data except skin lesions. All mice for the kidney pathology studies are N3 intercross littermates. Number of mice for the kidney pathology analyses: 5–6 for TdT+/+ and 7–9 for TdT−/−. Skin lesions were analyzed at 6 mo of age on 14 N3 mice and 19 N5 mice. The unpaired t test was used for kidney pathology and Fisher’s Exact test was used for skin lesions.
are present display the same enrichment for V\textsubscript{H}9252 sequences among the total V\textsubscript{H} sequences. TCR sequences were amplified with a V\textsubscript{H}9252 primer that equally amplified TCR genes, and both the TdT\textsuperscript{+/+} and TdT\textsuperscript{−/−} mice showed ˜55% representation of V\textsubscript{8} sequences among the total V\textsubscript{8} sequences (Table IV). As previously observed (22, 23), a wide variety of J\textsubscript{8} sequences were used. The CDR3 regions are an average of 0.8 aa shorter in length in the TdT\textsuperscript{−/−} mice (Table IV). Thus, despite a 30% reduction in the number of DN T cells in cervical LNs, the DN B220\textsuperscript{−} cells that were BrdU\textsuperscript{+} are plotted. Cells were stained as in Fig. 2, except that B cells were labeled with anti-CD19.

Table II. Organ weights for TdT\textsuperscript{+/+} and TdT\textsuperscript{−/−} MRL-Fas\textsuperscript{−/−} mice

<table>
<thead>
<tr>
<th></th>
<th>Spleen Weight</th>
<th>Cervical LN Weight</th>
<th>Mesenteric LN Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdT\textsuperscript{+/+}</td>
<td>883 ± 291</td>
<td>747 ± 229</td>
<td>670 ± 188</td>
</tr>
<tr>
<td>TdT\textsuperscript{−/−}</td>
<td>342 ± 56</td>
<td>391 ± 118</td>
<td>620 ± 109</td>
</tr>
<tr>
<td>\textit{p} values</td>
<td>0.01</td>
<td>0.059</td>
<td>0.419</td>
</tr>
</tbody>
</table>

* Spleen and LN weights are in milligrams, and are displayed as the mean ± SEM. Mice were 6 mo old and were from the N3 generation. Numbers of mice: five for TdT\textsuperscript{+/+} and nine for TdT\textsuperscript{−/−}. The unpaired \textit{t} test was used for all comparisons.

from the MRL-Fas\textsuperscript{−/−} TdT\textsuperscript{+/+} mice contained arginines, most of which were encoded by N regions.

**Analysis of DN B220\textsuperscript{+} T cells**

DN B220\textsuperscript{+} T cells have been shown to be enriched in V\textsubscript{8}3-expressing TCR (22–24). Because the DN B220\textsuperscript{+} T cells are reduced in the TdT\textsuperscript{−/−} MRL-Fas\textsuperscript{−/−} mice, we stained LN cells of 5-mo-old littermate mice with mAbs that react with V\textsubscript{8}1.1 + V\textsubscript{8}2.2, or that react with V\textsubscript{8}3. Cells were also stained with anti-CD3, anti-CD4, and anti-CD8, and were analyzed by FACS. We observed that DN B220\textsuperscript{+} T cells are similarly enriched in V\textsubscript{8}3 in the TdT-deficient mice as well as the TdT\textsuperscript{+/+} MRL-Fas\textsuperscript{−/−} mice (data not shown).

This enrichment in V\textsubscript{8}3 T cells was also observed in sequences derived from RNA from DN B220\textsuperscript{+} T cells from these same LN preparations. TCR sequences were amplified with a V\textsubscript{8} primer that equally amplifies all three V\textsubscript{8} genes, and both the TdT\textsuperscript{+/+} and TdT\textsuperscript{−/−} mice showed ˜55% representation of V\textsubscript{8}3 sequences among the total V\textsubscript{8} sequences (Table IV). As previously observed (22, 23), a wide variety of J\textsubscript{8} sequences were used. The CDR3 regions are an average of 0.8 aa shorter in length in the TdT\textsuperscript{−/−} mice (Table IV). Thus, despite a 30% reduction in the number of DN T cells in cervical LNs, the DN B220\textsuperscript{+} T cells that are present display the same enrichment for V\textsubscript{8}3 expression. Because they contain shorter CDR3 regions and use a variety of J\textsubscript{8} regions, this suggests that the CDR3 region of this unique subpopulation of cells is not as critical as the CDR1 or CDR2 regions of V\textsubscript{8}3 for recognition of their yet undescribed Ag(s), and thus may suggest an indirect rather than a direct effect of TdT deficiency on the reduced percentage of this subpopulation in the TdT\textsuperscript{−/−} mice.

**CDR3 regions of CD4\textsuperscript{+} T cell V\textsubscript{8} rearrangements**

RNA from splenic CD4\textsuperscript{+} T cells was amplified with V\textsubscript{8}8 and C\textsubscript{8} primers, and rearranged TCR \textsubscript{8} were sequenced. The average CDR3 length is 1.4 aa shorter in the TdT\textsuperscript{−/−} mice (Table V). Most of these sequences contained the acidic amino acids glutamic acid or aspartic acid, which have been associated with the CDR3 regions of T cells that can provide help for an anti-dsDNA response.
FIGURE 5. Circulating levels of polyclonal, anti-chromatin, anti-dsDNA IgG subclasses from N3 generation MRL-Fas<sup>+/−</sup> TdT<sup>+/+</sup> vs MRL-Fas<sup>+/−</sup> TdT<sup>−/−</sup> mice were determined by ELISA. Levels of IgM-RF with specificity for the different IgG subclasses are also shown. Serum concentrations from 6–8 mice/group were determined by ELISA after correlating to a precalibrated serum. *, p < 0.05.

Table III. V<sub>H</sub>J558 rearrangements from spleen<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>Average CDR3 Length</th>
<th>Total No. Sequences</th>
<th>No. Sequences with Arg in CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdT&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>10.2</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>TdT&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>9.1</td>
<td>38</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> DNA was made from spleen cells from MRL-Fas<sup>+/−</sup> N5 generation littersmates, and was amplified with V<sub>H</sub>J558 and J<sub>H</sub> primers. PCR products were cloned and sequenced. The number of sequences containing arginines (Arg) in CDR3 is shown.

Discussion

This study was stimulated by the findings of Conde et al. (13), who showed that TdT-deficient (NZB × NZW)<sub>F</sub><sub>1</sub> mice had increased lifespan and decreased incidence of GN, yet the TdT-deficient mice showed no decrease in titers of anti-dsDNA as compared with wild-type mice. Because anti-DNA autoantibodies, which are the hallmark of lupus, and which appear to play a key role in the pathology of systemic lupus erythematosus, often have increased numbers of arginines in their CDRs, one might have predicted that the absence of N regions might reduce the level or affinity of anti-dsDNA Abs, thus possibly decreasing symptoms of disease (26–29). Previous analysis of LPS-stimulated hybridomas from both B6 TdT-deficient mice and wild-type B6 mice showed that the TdT-deficient mice had a lower incidence than wild-type B6 mice of Abs that were polyreactive to several autoantigens, including ssDNA, although the frequency of monospecific autoantibodies, including anti-ssDNA, was the same for both groups of mice (12). The CDR3 lengths were 1–2 aa shorter in N region-lacking receptors from newborn mice (19, 30), and the larger CDR3 loops, particularly in IgGs, may be more likely to bind to a larger array of Ags, consistent with the increase in polyreactivity. The N region-containing hybridomas had a larger percentage that displayed higher avidity binding to ssDNA than the N region-lacking group, so this study would suggest that the N region-lacking repertoire in autoimmune-prone mice might be expected to be decreased in pathogenic anti-dsDNA Abs (12).

Thus, given that the (NZB × NZW)<sub>F</sub><sub>1</sub> TdT<sup>−/−</sup> mice had a much milder course of disease, it was surprising that they produced similar levels of anti-dsDNA to their wild-type counterparts. This suggested that some specificity other than anti-dsDNA autoantibodies was being affected by the lack of N regions. Therefore, we investigated the effect of TdT deficiency on several aspects of autoimmune disease in another lupus-prone strain of mice, MRL-Fas<sup>+/−</sup>, to determine whether we would obtain similar disease amelioration yet a lack of decrease in anti-dsDNA titers. We show here that in MRL-Fas<sup>+/−</sup> mice also, the onset of autoimmunity is delayed, and we observed a significant increase in longevity. However, in contrast to the (NZB × NZW)<sub>F</sub><sub>1</sub> mice, we observed a significant decrease in circulating anti-dsDNA. Also, we observed a significant decrease in IgG deposits in the kidney of the MRL-Fas<sup>+/−</sup> TdT<sup>−/−</sup> mice. The decrease in circulating anti-dsDNA levels is likely to be responsible for the decrease in IgG deposits in the kidney. In addition, we also observed a significant reduction in the generalized hypergammaglobulinemia characteristic of MRL-Fas<sup>+/−</sup> mice, and also a decrease in RF levels. However, we did observe some renal damage at the 6-mo time point that we studied, so the disease was likely delayed, not eliminated. The incidence of skin lesions, which are characteristic of human lupus and are also present in MRL-Fas<sup>+/−</sup> mice (21), was also reduced in the absence of TdT. Thus, the effect of TdT deficiency in both murine models was to slow down the course of disease, but the manner in which it affected various disease manifestations in both strains of mice is different.

Because TdT is expressed only in developing T and B cells, and its function is to add N region diversity to V-D-J junctions in CDR3, the decrease in pathology in the MRL-Fas<sup>+/−</sup> mice is likely to be due to a repertoire change in either B or T cells, or both. An obvious candidate is the anti-dsDNA B cell precursors. Not all anti-dsDNA Abs contain arginines, so this is not an absolute requirement, but arginines are far more prevalent in these Abs than in most other Abs (14, 31). Arginines in CDR1 and CDR2 arise
predominantly by somatic hypermutation and thus would not be affected by the absence of TdT. However, the arginines in CDR3 can also arise by N region addition or the use of alternative reading frames for the D regions (14). For Abs, the majority of DH regions are used in reading frame 1 in the absence of N regions, due to homology-directed recombination (4). However, the addition of N regions precludes homology-directed recombination, and thus the other two reading frames are observed at higher frequencies than in the fetal repertoire. The amino acids encoded by the three different reading frames do not encode any arginines (except for SP2/10), whereas the other reading frames do. Thus, TdT has a direct influence on the amino acid composition of CDR3, both in the N segment itself, and for the reading frame of the D segment.

These mechanisms, which act during the process of V(D)J recombination, could potentially increase the affinity of the primary repertoire of anti-dsDNA, and thus provide a precursor pool with a combination, could potentially increase the affinity for dsDNA, and thus provide a precursor pool with some minimal affinity for dsDNA. Twenty percent of all Vβ8 sequences in normal CDR3 to have a reasonable affinity for dsDNA, sufficient to be subsequently triggered by Ag, and undergo somatic hypermutation and become a high-affinity anti-dsDNA B cell. Twenty percent of all anti-dsDNA from MRL-Fas<sup>br</sup> TdT<sup>+/−</sup> and TdT<sup>−/−</sup> mice. cDNA was amplified with a Vβ8 and a Cβ primer, and the products were cloned and sequenced. The number of sequences with the acidic amino acids aspartic acid (Asp) or glutamic acid (Glu) are indicated.

<table>
<thead>
<tr>
<th>TdT&lt;sup&gt;+/−&lt;/sup&gt;</th>
<th>TdT&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of Vβ8 Sequences</td>
<td>18</td>
</tr>
<tr>
<td>No. Sequences with Asp or Glu in CDR3</td>
<td>18</td>
</tr>
<tr>
<td>Average No. of Asp or Glu/CDR3</td>
<td>1.78</td>
</tr>
<tr>
<td>Average CDR3 Length</td>
<td>10.0</td>
</tr>
<tr>
<td>% Vβ8,3</td>
<td>33</td>
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</tbody>
</table>

*CD4<sup>+</sup> T cells were isolated from spleens of 4-mo-old N5 generation MRL-Fas<sup>br</sup>/TdT<sup>+/−</sup> and TdT<sup>−/−</sup> mice. cDNA was amplified with a Vβ8 and a Cβ primer, and the products were cloned and sequenced. The number of sequences with the acidic amino acids aspartic acid (Asp) or glutamic acid (Glu) are indicated.

The effects of the TdT deficiency cover both a general reduction in hypergammaglobulinemia, and a decrease in the levels of autoantibodies characteristic of this disease. It may be that the decrease in levels of these autoantibodies is due to a reduction in the polyreactive, rather than monoreactive, Abs, as observed in the B6 TdT-deficient mice (12). Because the IgG2a anti-DNA Abs are decreased by approximately the same extent as the total IgG2a, it is not clear whether the anti-dsDNA levels are specifically reduced. In this regard, it may be that the absence of N regions in T cells that provide help for B cells causes a generalized decrease in the production of all serum Abs.

The autoantibody profile of the MRL-Fas<sup>br</sup> TdT<sup>−/−</sup> mice, although unlike that of the (NZB × NZW)<sub>F<sub>1</sub></sub> TdT<sup>−/−</sup> mice, was more similar to that of TdT-deficient B6-Fas<sup>br</sup> mice (36). In that study, which was published while we were analyzing our mice, B6-Fas<sup>br</sup> mice were made TdT deficient by backcrossing to TdT<sup>−/−</sup> mice, and the mice showed reduced levels of anti-ssDNA as compared with their TdT<sup>+/−</sup> counterparts. However, B6-Fas<sup>br</sup> mice do not make significant levels of anti-dsDNA Abs, which are the only pathogenic anti-DNA Abs, nor do they develop kidney or skin disease; MRL background genes are necessary for pathology. Splenic B cells from the B6-Fas<sup>br</sup> TdT<sup>+/−</sup> mice displayed a higher incidence of arginines in the IgH CDR3 regions as compared with the B6-Fas<sup>br</sup> TdT<sup>−/−</sup> mice, as has been shown previously for TdT<sup>+</sup> vs TdT<sup>−</sup> repertoires of normal mice. We found an even more pronounced difference in the frequency of arginines in the MRL-Fas<sup>br</sup> TdT<sup>+/−</sup> vs TdT<sup>−/−</sup> mice (Table IV). Similar to our finding that MRL-Fas<sup>br</sup> TdT<sup>−/−</sup> mice also had reduced levels of anti-chromatin Abs and IgM-RF compared with their wild-type littermates, the B6-Fas<sup>br</sup> TdT<sup>−/−</sup> mice were shown to have reduced titers of anti-histone components and of IgM-RF and IgG-RF. However, a significant difference is that the B6-Fas<sup>br</sup> mice did not show any decrease in their more modest hypergammaglobulinemia in the absence of TdT, whereas we observed an over 2-fold reduction in the massive hypergammaglobulinemia in the MRL-Fas<sup>br</sup> TdT<sup>−/−</sup> mice (36). Thus, autoantibody production and RF titers are decreased in both the autoimmune and nonautoimmune TdT-deficient mouse strains carrying the defective Fas<sup>br</sup> allele, but are not decreased in the (NZB × NZW)<sub>F<sub>1</sub></sub> autoimmune strain of mice.

In addition to effects on the primary B cell repertoire, T cells could also be affected by the absence of TdT, and may be responsible for some of the effects that were observed in these MRL-Fas<sup>br</sup> mice. T cell help is required for the somatic hypermutation and affinity maturation of high-affinity IgG anti-dsDNA Abs, and...
the production of mutated high-affinity Abs may be an initiating event in the pathogenesis of lupus. The specificity of the T cells that provide help for the anti-dsDNA B cells in vivo is not certain, but there is evidence that these T cells may be specific for basic DNA binding proteins such as histones. One study isolated T cells from (SWR × NZB)F1 mice that helped the production of anti-dsDNA Abs in vitro (25). Another group studied T cells specific for a DNA-binding basic peptide Fus1 (37). The T cells from both studies showed Vβ skewing and overrepresentation of Vβ8. TCRβ Vβ8 genes have a germline-encoded aspartic acid at position 99 that is often retained in CDR3, and Vβ8 are overrepresented in these clones. The CDR3 regions all had at least one acidic amino acid, some of which were generated by N nucleotides. Thus, these two studies would suggest that the T cells that may be required for providing T cell help in the production of pathogenic anti-dsDNA Abs in vivo might be decreased in relative frequency in N region-lacking populations. A different type of candidate Ag for the T cells required for lupus induction is peptides from VH genes used in the VDJ joining of immunoglobulin heavy chains. The major fraction of these peptides are enriched in charged amino acids (40). The structural characteristics of such TCR is unknown, but may be presumed to have complementary charged amino acids. Thus, these T cells also may be decreased in MRL-Fasbr TdT-deficient mice.

We amplified Vβ8 sequences from CD4+ T cells and observed that both TdT-deficient and TdT-containing CD4+ T cells had a high frequency of aspartic acids or glutamic acids in CDR3 (Table V). The majority of acidic residues in all cases were derived from the Vβ or Jβ sequences, although some of the acidic residues in the TdT+/− mice were N region encoded, and we did not note a slight decrease in the frequency of acidic residues in CDR3 of CD4+ T cells derived from TdT-deficient MRL-Fasbr mice.

These MRL-Fasbr TdT-deficient mice also showed a decrease in the incidence of skin lesions. Many cell types have been implicated in the production of skin lesions. Some studies have shown that IgG3 cryoglobulins from MRL-Fasbr mice, but not IgG3 Abs lacking this property, are capable of inducing skin lesions in mice (41). T cells are also clearly involved in the development of skin lesions. MRL-Fasbr mice that lack αβ T cells displayed delayed onset of skin lesions, whereas mice deficient in both αβ TCR and CD40 ligand (CD40L) developed a similar slow course of pathology as mice deficient only in αβ T cells, except that they did not develop skin disease at all (42). In contrast, CD40L deficiency alone led to less severe renal disease and less autoantibody production, but those mice had equivalent skin pathology to the wild-type MRL-Fasbr. Thus, αβ T cells may be involved in the development of skin disease by a CD40L-independent pathway, and CD40L interactions must also influence the development of skin disease in a non-αβ T cell pathway. How the lack of N regions affects these cells is not known.

MRL-Fasbr mice that lack CD4 T cells show greatly reduced autoantibodies and reduced incidence of GN (43). Because we observed a decrease in autoantibodies as well as a decrease in generalized hypergammaglobulinemia, we suggest that the lack of N regions is also affecting the CD4 T cells, which are required for the production of these autoantibodies, as well as for the somatic hypermutation required to generate high affinity, presumably pathogenic, anti-dsDNA Abs. Experiments are currently in progress to determine whether it is the T cell or B cell compartment, or both, which is critically affected by the absence of N regions, and which results in the increased lifespan and other aspects of disease amelioration in these TdT-deficient lupus-prone mice.

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References


