Selection of Anti-Double-Stranded DNA B Cells in Autoimmune MRL-\textit{lpr/lpr} Mice

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Abs to DNA and nucleoproteins are expressed in systemic autoimmune diseases, whereas B cells producing such Abs are edited, deleted, or inactivated in healthy individuals. Why autoimmune individuals fail to regulate is not well understood. In this study, we investigate the sources of anti-dsDNA B cells in autoimmune transgenic MRL-lpr/lpr mice. These mice are particularly susceptible to lupus because they carry a site-directed transgene, H76R that codes for an anti-DNA H chain. Over 90% of the B cells are eliminated in the bone marrow of these mice, and the few surviving B cells are associated with one of two Vκ editors, Vκ 38c and Vκ 21D. Thus, it appears that negative selection by deletion and editing are intact in MRL-lpr/lpr mice. However, a population of splenic B cells in the H76R MRL-lpr/lpr mice produces IgG anti-nuclear Abs, and these mice have severe autoimmune organ damage. These IgG Abs are not associated with editors but instead use a unique Vκ gene, Vκ 23. The H76R/Vκ 23 combination has a relatively high affinity for dsDNA and an anti-nuclear Ab pattern characteristic of lupus. Therefore, this Vκ gene may confer a selective advantage to anti-DNA Abs in diseased mice. The Journal of Immunology, 2006, 176:5183–5190.

Materials and Methods

Mice

The construction of H76R sd-tg mice was described previously (11). They were crossed to MRL-lpr/lpr (MRL-lpr), BALB/c, or C57BL/6 (B6) background for at least 10 generations. Mice were maintained and bred at the Oregon Health & Science University (Portland, OR) using stock mice BALB/c, B6, and MRL-lpr from The Jackson Laboratory. All animal care and procedures were conducted in accordance with the Animal Welfare Act.

Flow cytometric analysis

Single-cell suspensions of spleen and bone marrow were obtained by pressing spleens between glass slides and flushing femoral bones with a syringe, respectively. Subsequently, cells were absorbed of RBC by lysis with 0.14

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M NH₄Cl, followed by centrifugation over FBS and filtration through nylon mesh (74 μm. Small Parts). After washing with buffered media (PBS with 0.2% Na azide, 2% FBS), cells were harvested by centrifugation and resuspended to a 5 × 10⁶ cell/ml concentration. Cells (10⁶) were stained with combinations of the following anti-mouse Abs labeled with biotin, PE, FITC, or allophycocyanin: anti-B220 (RA3-6B2), anti-CD19 (1D3) (BD Pharmingen); and anti-IgM (polyclonal) and anti-IgD (polyclonal) (Southern Biotechnology Associates). For secondary staining, biotin-labeled Abs were coupled to Streptavidin-PerCP (BD Pharmingen). Stained cells were then analyzed for Ag expression using a FACSCalibur flow cytometry apparatus (BD Biosciences). Data were analyzed using CellQuest software.

**Generation of hybridomas**

Spleen cells from a 12-wk-old H76R/MRL-lpr mouse were divided into two parts: one was subjected to fusion with SP2/0 myeloma cells without manipulation, and the other was stimulated in vitro for 3 days with 20 μg/ml LPS (Sigma-Aldrich) before fusion. Established procedures were used in the generation of hybridomas (15).

**ELISA**

Ig isotype and concentration were measured by a solid phase ELISA as described previously (16). Plates were coated with goat-anti-mouse κ and λ (Southern Biotechnology Associates), incubated with sera or hybridoma supernatants, and developed with alkaline phosphatase-labeled anti-IgM or anti-IgG (Southern Biotechnology Associates). Ig concentration was calculated by comparing to titrations of purified mouse IgM or IgG standard. Plates were coated with goat anti-mouse Ig isotype and concentration were measured by a solid phase ELISA as described previously (17).

**Anti-nuclear Ab (ANA) detection**

Mouse sera or hybridoma supernatants diluted in buffer (1× PBS, 1% BSA, 0.02% NaN₃) to various concentrations (1/100, 1/500, 1/1000, 1/2500, 1/10000, 1/25000) were tested for ANA using an ANA test kit (Antibodies, Inc.). ANA slides were incubated with the sera for 45 min followed by washing with PBS. Subsequently, the slides were stained with anti-κ-FITC (Southern Biotechnology Associates) and visualized using a Nikon Junior fluorescent microscope. A homogeneous nuclear staining pattern was considered positive (17).

**Examination of Cκ deletion**

Cκ deletion was determined by PCR using a Cκ primer (RS-101) (18) and a degenerate Vκ primer, Vs (19), which amplifies at least 80% of the Vκ genes. The PCR conditions were the same as described previously (4).

**RT-PCR and sequencing**

RT-PCR and λ chain sequencing were conducted as described previously (20). CDNA was synthesized with a Cκ primer, and subsequently amplified with the Cκ and the degenerate Vκ primers. For 76R H chain sequencing, DNA was amplified directly from genomic DNA. The PCR products were subjected to automated, fluorescent DNA sequencing (ABI 377; Applied Biosystems) using nested Cκ or JH primer.

**Histology**

Fresh tissue was fixed in 10% neutral-buffered formalin for at least 24 h before being embedded in paraffin. Sections of 3-μm thickness were stained with H&E using standard procedure.

**Results**

The H76R transgene accelerates autoantibody production in MRL-lpr mice

Conversion to autoimmunity takes time. For example, in MRL-lpr mice, anti-dsDNA Abs do not appear until ~10 wk of age, and signs of kidney damage appear around 3–5 mo of age (21–24). In contrast, the H76R transgenic MRL-lpr mice develop anti-dsDNA Abs at 4–6 wk of age (Fig. 1A). In addition, the levels of anti-dsDNA Abs at 10–12 wk are higher in H76R/MRL-lpr mice than in non-tg MRL-lpr mice. This is also the case for V₅₆R in MRL-lpr (25) and in V₅₆R/B6.Sle1 mice (C. Mohan, unpublished observations). Thus, it appears that the addition of an anti-DNA tg to a lupus-susceptible mouse lowers the threshold for disease onset. A similar effect has been found in FcγRIIB-deficient mice on a B6 background and is thought to be due to loss of inhibitory effect of FcγRIIB on B cells (26). Why H76R affects the threshold is not clear: transgenic B cells could be precursors to IgG anti-DNAs, and/or they may play an indirect role, for example, as APCs.

In contrast to our findings, Mandik-Nayak et al. (24) have reported that V₅₆H, the precursor of V₅₆R and V₅₆, does not accelerate anti-dsDNA Ab production in MRL-lpr mice. This discrepancy is not due to a difference in Ab detection methods (ELISA vs ANA assay) because H76R/MRL-lpr mice also have an accelerated production of serum ANA (data not shown). Instead, it may be related to the status of the transgene: the VH₃H₉ used in the earlier experiments is a conventional transgene and therefore cannot undergo isotype switching, yet the most significantly increased anti-DNAs in H76R sd-tg mice are IgGs, which can only arise from isotype switching (Fig. 1). Another explanation is that the anti-DNA activity of V₅₆H is removed by L chain editing in the bone marrow (4), whereas very few L chains can veto anti-DNA activity of V₅₆R or V₅₆ due to their high affinity (27).

In addition to the high level of anti-dsDNA Abs, the H76R/MRL-lpr mice also develop severe glomerulonephritis, extensive lymphoid infiltrate, and vasculitis in the kidneys and lungs (Fig. 1B). However, the tissue pathology does not appear until 15–20 wk of age, similar to the non-tg MRL-lpr mice. This dis-
Many anti-dsDNA B cells are eliminated in the bone marrow of H76R/MRL-lpr mice

Even though H76R enhances and accelerates the production of anti-DNA Abs, 90% of the anti-DNA B cells are eliminated in the bone marrow of H76R MRL-lpr mice (Fig. 2A and Table I). B cells in the spleen of H76R/MRL-lpr mice are also decreased by 80% (Fig. 2B). It appears, for unknown reasons, that B cells in this tg are unable to exit and die in the bone marrow. They may be developmentally arrested and deleted (31), or may have undergone receptor deletion (M. Morden and M. Weigert, manuscript in preparation), or may have not received proper emigration signals from their BCRs (32).

Analyses of hybridomas from H76R/MRL-lpr and H76R/BALB/c mice

The few B cells that do exit bone marrow must be the source of anti-DNAs in H76R MRL-lpr mice. How do these B cells differ from those of the non-autoimmune mice? We compared hybridomas derived from LPS-activated splenic B cells of BALB/c and MRL-lpr H76R mice (Table II). Although most hybridomas from both types of mice have lost H76R tg and therefore are not informative, some B cells retain H tg. These B cells have restricted L chain usage: the editor Vx38c is used by 53% (27 of 51) of the H76R Abs from MRL-lpr mice and by 75% (39 of 52) of the Abs from BALB/c mice. The fact that the panels from these mice are similar suggests that central editing of anti-dsDNA B cells is intact in MRL-lpr mice.

Next, we have studied spontaneous hybridomas derived from unmanipulated splenic B cells of the same H76R/MRL-lpr mouse used to generate the LPS hybridomas. We consider these to be a surrogate for Ag-activated B cells. In this study, we obtained 58 hybridomas, of which 32 (55%) are IgG (Table II). This is in contrast to LPS hybridomas which are 93% IgM. Importantly, 13 of the 32 IgG mAbs retain the H76R tg, and nine of them bind dsDNA with relatively high affinity and exhibit an ANA pattern that is typical of lupus (Table III and Fig. 3). Eight of the nine anti-dsDNA IgGAbs are encoded by Vx23 and one by Vx19 (Table III). Such a high representation of Vx23 is surprising because many L chains can sustain DNA binding (20, 25).

Vx23 hybridomas may result from clonal expansion of one or a few B cells. However, this is very unlikely. These Vx23 mAbs are encoded by two different Vx23 genes: three by Vx23-43 and five by Vx23-45 (Table III). In addition, the three Vx23-43 hybridomas have different Vκ-Jκ junctions (Fig. 4A) and therefore, are the products of three different B cells. The five Vx23-45 hybridomas use three different Jκs: two Jκ2, one Jκ4, and two Jκ5. They too must represent at least three independent clones. The two Vx23-45/Jκ5 hybridomas (123 and 201) are probably unrelated based on mutation frequency: clone 123 has five mutations in the VH region, whereas clone 201 has none (Fig. 4B). The two

Table I. Frequencies and absolute numbers of bone marrow B cells in H76R sd-tg mice

<table>
<thead>
<tr>
<th></th>
<th>B220⁺ IgM⁺</th>
<th>B220⁺ IgM⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(%)</strong></td>
<td>No. (×10⁵)</td>
<td><strong>(%)</strong></td>
</tr>
<tr>
<td>B6</td>
<td>21 ± 6.7</td>
<td>21 ± 6.6</td>
</tr>
<tr>
<td>76R/B6</td>
<td>3.0 ± 1.5</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>MRL-lpr</td>
<td>12 ± 4.7</td>
<td>9.1 ± 5.0</td>
</tr>
<tr>
<td>76R/MRL-lpr</td>
<td>1.6 ± 1.2</td>
<td>1.5 ± 1.3</td>
</tr>
</tbody>
</table>

*Mice were 8–12 wk old and were crossed to B6 or MRL-lpr background for at least 10 generations.

*Percentages of bone marrow B cells were calculated using the cells in the lymphocyte gate, and the absolute numbers were deduced from the total bone marrow cells of both femurs. Data are presented as mean ± SD. B220⁺ IgM⁺ cells represent immature B and mature B cells, and B220⁺ IgM⁻ cells include pro-B and pre-B cells.

FIGURE 2. Elimination of anti-dsDNA B cells in the bone marrow and spleen of H76R/MRL-lpr mice. Bone marrow (A) and spleen (B) cells from B6, H76R/B6, MRL-lpr, and H76R/MRL-lpr mice were stained with Abs against B220, CD19, and IgM. The percentages of B220⁺ IgM⁺ or CD19⁺ IgM⁺ cells were determined using the cells in the lymphocyte gate. Eight to 15 mice of each genotype were tested, and the mice were 10–15 wk old.
Table II. Characterization of hybridomas from H76R/BALB/c and H76R/MRL-lpr mice

<table>
<thead>
<tr>
<th>Clones</th>
<th>LPS H76R/BALB/c</th>
<th>LPS H76R/MRL-lpr</th>
<th>Spont H76R/MRL-lpr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM/allele (%)</td>
<td>DNA+ (%)</td>
<td>IgM/allele (%)</td>
</tr>
<tr>
<td>167</td>
<td>136 (98%)</td>
<td>106 (45%)</td>
<td>58 (52%)</td>
</tr>
<tr>
<td>135</td>
<td>3 (2%)</td>
<td>30 (55%)</td>
<td>1 (28%)</td>
</tr>
<tr>
<td>198</td>
<td>52 (37%)</td>
<td>41 (48%)</td>
<td>19 (28%)</td>
</tr>
<tr>
<td>201</td>
<td>198 (63%)</td>
<td>14 (55%)</td>
<td>6 (30%)</td>
</tr>
</tbody>
</table>

Vκ gene usage and dsDNA binding among H76R-positive clones

<table>
<thead>
<tr>
<th>Clones</th>
<th>Vκ</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All DNA+</td>
<td>All DNA+</td>
<td>All DNA+</td>
<td>All DNA+</td>
<td>All DNA+</td>
<td>All DNA+</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>51</td>
<td>40</td>
<td>1</td>
<td>1</td>
<td>46</td>
<td>28</td>
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<td>135</td>
<td>Vκ23</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>201</td>
<td>Vκ20</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>198</td>
<td>Vκ19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>201</td>
<td>Vκ19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>167</td>
<td>Vκ19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>135</td>
<td>Vκ19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>198</td>
<td>Vκ19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>201</td>
<td>Vκ19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>167</td>
<td>Vκ23</td>
<td>48</td>
<td>28</td>
<td>5</td>
<td>2</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>135</td>
<td>Vκ23</td>
<td>25</td>
<td>24</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>198</td>
<td>Vκ23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>201</td>
<td>Vκ23</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: The hybridomas were derived from LPS-stimulated splenic B cells of a 12-wk-old H76R/MRL-lpr mouse, and the data were adapted from Ref. 11.

Vκ23-45/Jκ2 hybridomas (104 and 229) may be related because they both have a histidine (His) at the Vκ-Jκ junction and identical, mutated VH sequences (Fig. 4). In summary, seven of eight Vκ23 IgG hybridomas are of independent origin, and expansion of one or a limited number of B cells does not account for the high frequency of Vκ23 in these anti-DNA Abs.

Vκ23 appears to have a selective advantage over other anti-DNA Abs. Vκ23 Abs may arise early or late in H76R MRL-lpr mice. Because either possibility may require extended rearrangement, we examined Cκ deletion in Vκ23 clones (Fig. 5). Several rounds of secondary rearrangement will ultimately delete Cκ by rearrangement of Vκ to the RS sequence located downstream of Cκ (C-RS) or, less frequently, by combining the VJ intron RS (I-RS) with the C-RS (Fig. 5) (18). In five of the nine anti-dsDNA IgGs, Cκ is deleted (Table III), and because the PCR used only detects the rearrangement of Vκ to C-RS (~70% of the Cκ deletion events), seven (78%) of the IgG hybridomas may have inactivated one κ allele via Cκ deletion. After an allele is deleted, the second κ allele may be rearranged to express a new Vκ gene such as Vκ23 or Vκ19 (Fig. 5). Because hardly any non-dsDNA-binding hybridomas (2 of 77) have Cκ deletion (data not shown), such secondary rearrangement appears peculiar to IgG anti-DNAs.

If secondary L chain rearrangement takes place during the Ag-driven phase of immune response, the new VL should lag behind VH in the frequency of mutation. We have reported one such example (33). In this study, we find VH mutation frequency exceeds that of VL mutation: six of eight Vκ23-associated hybridomas have VH mutation, but only two have VL mutation (the shared Arg-to-Lys change in Vκ23-43 sequences most likely represents allelic variation between mouse strains rather than mutation). Moreover,

Table III. Characterization of spontaneous anti-dsDNA IgG hybridomas from a H76R/MRL-lpr mouse

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>Isotype</th>
<th>VH</th>
<th>dsDNA</th>
<th>ANA</th>
<th>Vκ</th>
<th>Jκ</th>
<th>Cκ-del</th>
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</thead>
<tbody>
<tr>
<td>167</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vκ23-43</td>
<td>Jκ2</td>
<td>+</td>
</tr>
<tr>
<td>135</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vκ23-43</td>
<td>Jκ2</td>
<td>–</td>
</tr>
<tr>
<td>198</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vκ23-43</td>
<td>Jκ2</td>
<td>–</td>
</tr>
<tr>
<td>201</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vκ23-45</td>
<td>Jκ5</td>
<td>+</td>
</tr>
<tr>
<td>123</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vκ23-45</td>
<td>Jκ5</td>
<td>–</td>
</tr>
<tr>
<td>251</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vκ19-15</td>
<td>Jκ4</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: The hybridoma clones are derived from a H76R/MRL-lpr mouse as described in Table II. The intensity of dsDNA binding is based on OD405 values: 0-0.3, 0.4-0.6, 0.7-1.0, and above 1.5. The Vκ and Jκ genes are determined by sequencing analysis, and Cκ deletion is determined by a Cκ-del PCR (see Materials and Methods).
consistent with dsDNA binding, and the sera from H76R/B6 mice have no lpr
(see text) and 20 serum samples from each type of mouse.

Discussion

Systemic lupus erythematosis is a polygenic disease (39–42). Several gene loci have been identified to contribute to various facets of disease process in New Zealand mice (43, 44). In MRL-lpr mice, both lpr and MRL host genes are required for disease development (45–47). Lpr represents a Fas deletion mutant (48, 49), and, therefore, it may exacerbate autoimmunity by preventing apoptosis of self-reactive lymphocytes. In H76R/MRL-lpr mice, however, anti-dsDNA B cells are efficiently eliminated in the bone marrow, indicating that Fas-Fas ligand pathway is not required in maintaining central tolerance. This is in agreement with previous findings that regulation of anti-HEL and anti-H-2k B cells is intact in the absence of Fas (6–8). However, despite good central tolerance, the H76R/MRL-lpr mice develop high titers of ANA, marked lymphadenopathy, and autoimmune organ damage. The Lpr mutation is required for these manifestations because they are not seen in H76R/MRL-+ mice (data not shown). The Lpr mutation may impair activation-induced apoptosis of peripheral lymphocytes (50–52). How the MRL host genes affect the tolerance breakdown is not known. They may contribute to B cell hyperactivity (53), accelerated differentiation of Ab-forming cells (54), cytokine overproduction (55), or inefficient clearance of self-Ags (56).

Studies on LPS-derived hybridomas from Vh3H9 transgenic and its derivatives (Vh68, Vh46, and Vh56R76R) have shown that there are several L chains that can edit DNA binding. These editors have more aspartates in CDRs than other L chains (11), a correlation which suggests that these acidic residues may interfere with the interaction between H chain Arg and DNA. However, there are differences in the editor distribution among the variaties of 3H9 Vh transgensics: the representation of Vk21/13 ranges from 64% for Vh3H9 to 26% for Vh66 and 0% for Vh56R (11, 57), whereas the representation of Vx21D (Vx21-4) is 78% for Vh56R (11). We attribute the differences in editor usage in BALB/c transgensics to their efficiencies of editing H chains with different Arg content and location. Thus, Vh66 can only be edited by Vx21D or Vx20, editors with the highest content of aspartate (11); but Vh3H9 and Vh68 can be edited by these and other L chains.

![FIGURE 3. ANA staining of sera and hybridoma supernatants from H76R/MRL-lpr mice. The sera and IgG hybridoma supernatants from H76R/MRL-lpr mice show a homogeneous nuclear staining pattern consistent with dsDNA binding, and the sera from H76R/B6 mice have no staining. The sera were used at 1/500 dilution and the supernatants at 1 μg/ml Ig concentration. Shown here are representatives of nine IgG anti-DNA hybridomas (see text) and 20 serum samples from each type of mouse.](http://www.jimmunol.org/)

![FIGURE 4. Amino acid sequences of the Vκ and VH genes of the Vκ23-expressing IgG hybridomas derived from an H76R/MRL-lpr mouse. The Vκ23 sequences (A) are compared with the germline Vκ23-43 and Vκ23-45 sequences (69), and the VH sequences (B) are compared with the VH3H9/56R/76R sequence (11). The His (H) and lysine (K) residues unique to Vκ23 are highlighted in bold. Sequence identities are indicated with dashes. Replacement mutations are shown in upper cases, and silent mutations are in lower cases. The CDRs are defined according to Kabat et al. (35).](http://www.jimmunol.org/)

Vh mutations of these Vκ23 Abs show evidence of selection by DNA because the mutations are concentrated in CDR2 and two introduce Arg (Fig. 4). Four Vh sequences share the Gly-to-Val mutation at position 55 in CDR2; and this site, TGG, may be a mutational hot spot similar to the reported hot spot sequence TAG (the underlined base is the target of mutation) (34). In summary, the difference in the accumulation of mutations suggests that the Vκ23 genes may be generated after H chain mutation has begun. An argument for early selection is that three of the eight Vκ23 Abs in our panel have a His at the V-J junction (codon 96). The codon for His at this position is created by an unusual intracodon splice between the 5' end "C" of Vκ23 and the "AT" at the Tyr codon in Jκ2 (Fig. 6). This is a rare event: of 696 Vκ-Jκ junctions, only 12 (1.7%) had His at position 96 (35). The recurrence of His96, a residue that might interact with DNA, in the Vκ23 Abs suggests its importance in Ag binding. It appears that both early (His96) and late (Arg mutation) selections contribute to the predominance of Vκ23 among IgG anti-DNA Abs. Therefore, as with other pathogenic Abs found in systemic lupus patients and animals (12, 36–38), the Vκ23 Abs from H76R/MRL-lpr mice are selected for and driven by DNA.
the stage at which Vκ38c is distal to Vκ23 on the chromosome, and both are in reverse orientation (69). This genomic organization allows a precursor-progeny relationship between Vκ38c and Vκ23. The 3′ Ck recombination sequence (C-RS) and the intrinsic RS (I-RS) are shown as shaded boxes. In the bone marrow, the first allele may undergo multiple rounds of rearrangement until an editor, such as Vκ38c, is found. In the periphery, further rearrangement results in Ck deletion via one of the two RS’s. Inactivation of this allele leads to rearrangement of the second allele and subsequent expression of Vκ23.

H76R B cells from BALB/c mice have an unusual editor repertoire consisting of nearly all Vκ38c (Table II). This is unexpected because H76R/Vκ38c binds DNA (Table II). Why is Vκ38c preferred as a 76R editor? Perhaps other editors are negatively selected for other reasons such as less than optimal heavy/light fit; alternatively, Vκ38c may have a specificity that is positively selected. Indeed, H76R/Vκ38c Ab binds a variety of self and foreign Ags, including DNA, phosphatidylserine, phosphocholine, and albumin (M. Weigert, unpublished observation). It has been suggested that low-affinity anti-self B cells are naturally selected (58), but their origin has not been adequately explained. In this study, we provide an explanation for the etiology of polyreactive Abs, namely editing of anti-DNA B cells. This edited population is, of course, a major component of the B cell repertoire in anti-DNA transgenics; however, even normal individuals are thought to have a significant frequency (~60%) of anti-DNA precursors (59). Thus, the frequency and etiology of polyreactive Abs as described in this study may apply to all individuals, not just anti-DNA transgenics.

Although Vκ38c is the most frequent L chain of the IgM anti-DNA Abs in H76R/MRL-lpr mice, it is rarely found among IgG Abs. Instead, Vκ23 is the most frequent IgG-associated L chain. The shift in L chain usage is undoubtedly due to Ag selection, but in this study may apply to all individuals, not just anti-DNA Abs. Instead, Vκ38c binds DNA (Table II). Why is Vκ38c preferred as a 76R editor? Perhaps other editors are negatively selected for other reasons such as less than optimal heavy/light fit; alternatively, Vκ38c may have a specificity that is positively selected. Indeed, H76R/Vκ38c Ab binds a variety of self and foreign Ags, including DNA, phosphatidylserine, phosphocholine, and albumin (M. Weigert, unpublished observation). It has been suggested that low-affinity anti-self B cells are naturally selected (58), but their origin has not been adequately explained. In this study, we provide an explanation for the etiology of polyreactive Abs, namely editing of anti-DNA B cells. This edited population is, of course, a major component of the B cell repertoire in anti-DNA transgenics; however, even normal individuals are thought to have a significant frequency (~60%) of anti-DNA precursors (59). Thus, the frequency and etiology of polyreactive Abs as described in this study may apply to all individuals, not just anti-DNA transgenics.

Although Vκ38c is the most frequent L chain of the IgM anti-DNA Abs in H76R/MRL-lpr mice, it is rarely found among IgG Abs. Instead, Vκ23 is the most frequent IgG-associated L chain. The shift in L chain usage is undoubtedly due to Ag selection, but at the stage at which Vκ23 arises could be central or peripheral. If the IgG Vκ23 B cells come from rare Vκ23-expressing IgM precursors that have escaped central tolerance, then they should show evidence of clonal expansion. However, the eight Vκ23 IgG anti-DNA mAbs are derived from at least seven independent B cell clones, and are thus not the products of clonal expansion. In contrast, our data suggest that peripheral secondary L chain rearrangement may generate at least some Vκ23 B cells in H76R/MRL-lpr mice. First, >50% of the IgG anti-dsDNA B cells have Ck deletion, a sign of extended rearrangement. Second, each of the six mutated hybridomas (clone no. 104, 123, 229, 135, 167, and 198) has more mutations in Vκ11 than in Vκ12 (Fig. 4). Clone no. 123 is the extreme: its Vκ11 has accumulated five mutations, and its Vκ has none. Assuming that Vκ11 and Vκ12 mutations are initiated at the same time during an immune response, this finding suggests that Vκ23 has rearranged in the periphery.

Eilat and colleagues (60, 61) have made similar discoveries in the lupus-prone New Zealand Black (NZB)/New Zealand White (NZW) mice expressing an anti-DNA H chain sd-tg (VH12D42). They show that high-affinity anti-DNA Abs are generated by secondary L chain gene rearrangements in NZB/NZW but not in non-autoimmune C57BL/6 × BALB/c F1 mice. Strikingly, as in our system, a single Vκ gene (VκRF) is repeatedly used by almost all the IgG anti-DNA Abs, indicating a strong Ag selection. The secondary rearrangement of the VκRF appears to occur in the bone marrow at the immature B stage, but our current study and a previous report (33) suggest that L chain re-editing may take place in the periphery during or after somatic mutation in autoimmune MRL-lpr mice. This variation may be attributed to the different affinity/specificity of the VH76R and VH12D42 anti-DNA Abs. Or, the defect in immune tolerance is somewhat different in MRL-lpr and NZB/NZW mice. Regardless of the timing of its occurrence, L chain secondary rearrangement represents a common mechanism in both strains of autoimmune mice, and potentially in systemic lupus erythematosus patients, for generating high-affinity autoantibodies.

The overrepresentation of Vκ23 in H76R/MRL-lpr mice is surprising because more variety of Vκs would be expected. Studies on Vκ3H9 and Vκ56R transgenics have shown that many L chains besides Vκ23 can yield anti-dsDNAs in combination with the tg H chains (20, 25); however, the bias toward Vκ23 cannot be attributed to expansion of one or a few clones (see above). Vκ23 overrepresentation is common among anti-DNAAs in different autoimmune mice (33, 62–67). In contrast, Vκ23 is one of the least frequently used Vκ genes in non-autoimmune B cells: of 1040 Vκ sequences compiled by Kabat et al. (35), only 12 (1%) are Vκ23, and only four of these are round non-self Ags. Recently, Mohan and colleagues (68) compiled a L chain database grouping the Abs into

**FIGURE 5.** Proposed model of receptor editing and re-editing in H76R/MRL-lpr mice. The two alleles of germline L chain locus are shown on top. It has been demonstrated that Vκ38c is distal to Vκ23 on the chromosome, and both are in reverse orientation (69). This genomic organization allows a precursor-progeny relationship between Vκ38c and Vκ23. The 3′ Ck recombination sequence (C-RS) and the intrinsic RS (I-RS) are shown as shaded boxes. In the bone marrow, the first allele may undergo multiple rounds of rearrangement until an editor, such as Vκ38c, is found. In the periphery, further rearrangement results in Ck deletion via one of the two RS’s. Inactivation of this allele leads to rearrangement of the second allele and subsequent expression of Vκ23.

**FIGURE 6.** Predicted Vκ-Jκ joinings of the five Vκ23-Jκ2 hybridomas. Partial nucleotide sequences of the Vκ23 of MRL-lpr origin (67) and Jκ2 are shown. Possible V-J joining that results in His<sup>60</sup> (clones 104, 198, and 229) is shown in solid line; V-J joining of Tyr<sup>60</sup> (clone 135) is shown in broken line, and V-J joining leading to Leu<sup>60</sup> (clone 167) is shown in dotted line.
ANA and non-ANA panels of nonredundant sequences. In this study, again, Vx23 is associated with anti-DNAs more frequently than with non-ANAs. Therefore, Vx23 must have properties that enhance DNA binding. A comparison of Vx23 to all other Vx sequences reveals two unique residues: His at L41 and Lys at L49, both of which are basic residues in FW2 (Fig. 4A). These two residues together with other three basic residues in the same region (His at L34, Lys at L39, and Arg at L45) yield a highly positively charged (pl 10.7) 16-aa region that might interact with DNA.

In summary, anti-DNAs appear to be under both negative and positive selection in MRL-lpr mice. Newly generated anti-DNA B cells in bone marrow are negatively regulated by receptor editing and deletion, most likely due to interaction with DNA-containing self-Ags in the blebs of apoptotic cells. The preferred editors are those that can reduce DNA-binding affinity while conferring polyreactivity, seem to be positively selected to emigrate to the periphery. Here, they can regain their initial anti-DNA activity via somatic mutation and/or secondary L chain rearrangement in an immune response to DNA Ags. Some of the peripherally generated B cells, such as those that express Vx23, are selected to dominate the IgG anti-DNA repertoire.

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