Selection of Anti-Double-Stranded DNA B Cells in Autoimmune MRL- *lpr/lpr* Mice

Ching Chen, Hui Li, Qi Tian, Michael Beardall, Yang Xu, Nina Casanova and Martin Weigert

*J Immunol* 2006; 176:5183-5190; doi: 10.4049/jimmunol.176.9.5183

http://www.jimmunol.org/content/176/9/5183

---

**References**

This article cites 67 articles, 36 of which you can access for free at:

http://www.jimmunol.org/content/176/9/5183.full#ref-list-1

---

**Why The JI?** Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

---

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

---

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

---

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Selection of Anti-Double-Stranded DNA B Cells in Autoimmune MRL-lpr/lpr Mice

Ching Chen,2* Hui Li,† Qi Tian,* Michael Beardall,* Yang Xu,* Nina Casanova,* and Martin Weigert†

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, Vκ38c and Vκ21D. 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, Vκ23. The H76R/Vκ23 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.

B cell tolerance to self-Ags is established in the bone marrow by receptor editing (1–5), but whether a failure to edit leads to autoimmunity is uncertain. Anti-hen egg lysozyme (HEL)3 and anti-H-2k transgenic lpr/lpr mice show little, if any, loss of tolerance to anti-HEL and anti-H-2k B cells (6, 7). However, these mice do produce anti-DNA Abs. Because tolerance to HEL and H-2k is maintained but tolerance to DNA is not, it seems that the defect in lpr/lpr mice is specific for certain Ags such as DNA (8). To study this defect, we established a series of site-directed tg (sd-tg) mouse models with VH genes coding for anti-DNA Abs of varying affinity (9–11). They are referred to as Vκ3H9 (the VH of a prototypic anti-DNA Ab originally isolated from a diseased MRL-lpr mouse) (12), and Vκ3H9/56R and Vκ3H9/56R/76R according to the position of arginine (Arg) substitutions introduced by in vitro mutagenesis. The Vκ3H9/56R is derived from 3H9 by adding an Arg residue at codon 56 of VH, and the 3H9/56R/76R H chain has an additional Arg at codon 76. These changes were made because Arg is a key residue for DNA binding, and many lupus-associated anti-DNAs have somatic mutations to Arg at these positions (13). The 56R increases the affinity for dsDNA by 10-fold, and the 56R/76R combination increases the affinity by 100-fold over that of 3H9 (13). Another consequence of the Arg additions is that fewer L chains are capable of editing the DNA binding activity of high-affinity VH3H9/56H9 variants. In the case of 56R, B cells use predominantly one κ L chain, Vκ21D, and one A L chain, Ax, as editors (11, 14). In the case of 56R/76R, no L chains completely veto DNA binding. The Vκ3H9/56R B cells also express dual receptors with one sustaining the autoreactivity and another that does not. These dual receptor B cells are confined to the splenic marginal zone, probably preventing these B cells from undergoing a secondary immune response (10). Each of these models provides a unique opportunity to study different aspects of B cell tolerance and how it might be broken in disease.

In this study, we analyze B cell regulation in autoimmune MRL-lpr mice with the Vκ3H9/56R/76H (H76R) sd-tg. We show that the majority of the anti-dsDNA B cells die in the bone marrow in both non-autoimmune and the autoimmune H76R mice, but a few B cells escape to the periphery by either VH replacement or L chain editing. The difference between the healthy and the MRL-lpr H76R mice is that the latter have IgG anti-dsDNA Abs and develop autoimmune disease. The Vκ genes associated with these IgG anti-DNAs arise after multiple rearrangements and are not editors. In addition, the Vκ regions of these IgG Abs have many CDR mutations, and they exhibit relatively high affinity for DNA. Therefore, it would appear that breakdown of tolerance in MRL-lpr mice is associated with an abnormal immune response that selects and, perhaps, creates (via somatic mutation and editing) high-affinity anti-self Abs.

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-lprfs 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

Copyright © 2006 by The American Association of Immunologists, Inc.

0022-1767/06/$02.00

Received for publication December 5, 2005. Accepted for publication February 8, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by grants from National Institutes of Health (AI-50818), The PEW Charitable Trust, and Cancer Research Institute (to C.C.), National Institutes of Health Grant GM-20964, and the Lupus Research Foundation (to M.W.). 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-lprfs 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

Abbreviations used in this paper: HEL, hen egg lysozyme; sd-tg, site-directed tg; Arg, arginine; ANA, anti-nuclear Ab; His, histidine; NZB, New Zealand Black; NZW, New Zealand White.
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 x 10^5 cell/ml concentration. Cells (10^6) 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). Anti-dsDNA binding was conducted by comparing to titrations of purified mouse IgM or IgG standard supernatants, and developed with alkaline phosphatase-labeled anti-IgM or anti-IgG (Southern Biotechnology Associates). Anti-dsDNA binding was conducted by a two-step solution phase ELISA as described previously (17).

**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 maturation, 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 (Southern Biotechnology Associates). Anti-dsDNA binding was conducted by a two-step solution 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, 1/12500) were tested for ANA using a 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(J) 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 κ L chain sequencing were conducted as described previously (20). CDNAs were 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 Vv56R in MRL-lpr (25) and in Vv56R/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 FcyRIIB-deficient mice on a B6 background and is thought to be due to loss of inhibitory effect of FcyRIIB 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 Vv76R, the precursor of Vv56R and Vv76R, 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 Vv3H9 used in the bone marrow (4), whereas very few L chains can veto anti-DNA activity of Vv56R or Vv76R 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. It has been shown that IgG anti-DNAs play a role in lupus nephritis (22, 28), but the basis of their pathogenicity is still elusive. Deposition of DNA-anti-DNA immune complexes in the glomeruli may initiate tissue inflammation, or anti-DNAs may bind to nucleosomes or DNA-histone compounds present on the cell surface (29). Furthermore, anti-DNAs may directly bind to glomerular structure via cross-reactivity (30). The delayed tissue damage may result from an initial low affinity of anti-DNA Abs, or from a lag in acquisition of new specificities such as those toward nucleosomes. Abs with high DNA-binding affinity and/or new specificities may be produced
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 Vκ38c 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 IgG Abs are encoded by Vκ23 and one by Vκ19 (Table III). Such a high representation of Vκ23 is surprising because many L chains can sustain DNA binding (20, 25).

Vκ23 hybridomas may result from clonal expansion of one or a few B cells. However, this is very unlikely. These Vκ23 mAbs are encoded by two different Vκ23 genes: three by Vκ23-43 and five by Vκ23-45 (Table III). In addition, the three Vκ23-43 hybridomas have different Vκ-Jκ junctions (Fig. 4A) and, therefore, are the products of three different B cells. The five Vκ23-45 hybridomas use three different Jκs: two Jκ2, one Jκ4, and two Jκ5. They too must represent at least three independent clones. The two Vκ23-45/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>Mice</th>
<th>n</th>
<th>B220+ IgM+ (%)*</th>
<th>No. (×10⁶)*</th>
<th>B220+ IgM+ (%)*</th>
<th>No. (×10⁶)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>6</td>
<td>21 ± 6.7</td>
<td>21 ± 6.6</td>
<td>30 ± 10</td>
<td>41 ± 11</td>
</tr>
<tr>
<td>76R/B6</td>
<td>7</td>
<td>3.0 ± 1.5</td>
<td>2.8 ± 1.3</td>
<td>32 ± 10</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>MRL-lpr</td>
<td>7</td>
<td>12 ± 4.7</td>
<td>9.1 ± 5.0</td>
<td>22 ± 7.5</td>
<td>21 ± 6.6</td>
</tr>
<tr>
<td>76R/MRL-lpr</td>
<td>12</td>
<td>1.6 ± 1.2</td>
<td>1.5 ± 1.3</td>
<td>22 ± 7.5</td>
<td>19 ± 6.8</td>
</tr>
</tbody>
</table>

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

b Percentages of bone marrow B cells were calculated using the cells in the lymphocyte gating, 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.
Characterization of hybridomas from H76R/BALB/c and H76R/MRL-lpr mice

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>Isotype</th>
<th>V_H</th>
<th>dsDNA</th>
<th>ANA</th>
<th>V_K</th>
<th>J_K</th>
<th>Ce-del</th>
</tr>
</thead>
<tbody>
<tr>
<td>167</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk2</td>
<td>+</td>
</tr>
<tr>
<td>135</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk2</td>
<td>−</td>
</tr>
<tr>
<td>198</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk2</td>
<td>−</td>
</tr>
<tr>
<td>104</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk2</td>
<td>+</td>
</tr>
<tr>
<td>229</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk2</td>
<td>+</td>
</tr>
<tr>
<td>98</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk4</td>
<td>+</td>
</tr>
<tr>
<td>201</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk5</td>
<td>−</td>
</tr>
<tr>
<td>123</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx23-43</td>
<td>Jk5</td>
<td>−</td>
</tr>
<tr>
<td>251</td>
<td>IgG</td>
<td>76R</td>
<td>++++</td>
<td>+</td>
<td>Vx19-15</td>
<td>Jk4</td>
<td>+</td>
</tr>
</tbody>
</table>

The hybridoma clones are derived from a H76R/MRL-lpr mouse as described in Table II. The intensity of dsDNA binding is based on OD_{405} values: +, OD 0.3–0.6; ++, OD 0.6–1.0; +++, OD 1.0–1.5; ++++, OD > 1.5. The Vx and Jx genes are determined by sequencing analysis, and Ce deletion is determined by a Ce_{del} PCR (see Materials and Methods).

Vx23-45/Jx2 hybridomas (104 and 229) may be related because they both have a histidine (His) at the Vx-Jx junction and identical, mutated V_H sequences (Fig. 4). In summary, seven of eight Vx23 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 Vx23 in these anti-dsDNA Abs.

Vx23 appears to have a selective advantage over other anti-dsDNA Abs. Vx23 Abs may arise early or late in H76R MRL-lpr mice. Because either possibility may require extended rearrangement, we examined Dx deletion in Vx23 clones (Fig. 5). Several rounds of secondary rearrangement will ultimately delete Dx by rearrangement of Vx to the RS sequence located downstream of Cx (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, Cx was deleted (Table III), and because the PCR used only detects the rearrangement of Vx to C-RS (~70% of the Cx deletion events), seven (78%) of the IgG hybridomas may have inactivated one κ allele via Cx deletion. After an allele is deleted, the second κ allele may be rearranged to express a new Vx gene such as Vx23 or Vx19 (Fig. 5). Because hardly any non-dsDNA-binding hybridomas (2 of 77) have Cx deletion (data not shown), such secondary rearrangement appears peculiar to IgG anti-dsDNA Abs.

If secondary L chain rearrangement takes place during the Ag-driven phase of immune response, the new V_L should lag behind V_H in the frequency of mutation. We have reported one such example (33). In this study, we find V_H mutation frequency exceeds that of V_L mutation: six of eight Vx23-associated hybridomas have V_H mutation, but only two have V_L mutation (the shared Arg-to-Lys change in Vx23-43 sequences most likely represents allelic variation between mouse strains rather than mutation). Moreover,
VH mutations of these Vk23 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 Vk23 genes may be generated after B cell chain mutation has begun.

An argument for early selection is that three of the eight Vk23 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 Vk23 and the “AT” from the Tyr codon in Jκ2 (Fig. 6). This is a rare event: 696 Vk-κ-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 Vk23 Abs suggests its importance in Ag binding. It appears that both early (His96) and late (Arg mutation) selections contribute to the predominance of Vk23 among IgG anti-DNA Abs. Therefore, as with other pathogenic Ab found in systemic lupus patients and animals (12, 36–38), the Vk23 Abs from H76R/MRL-lpr mice are selected for and driven by DNA.

**Discussion**

Systemic lupus erythematosus 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, antidiDNA 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-H2k 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 (VH3Germ line, VH56R, 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 Args and DNA. However, there are differences in the editor distribution among the varieties of VH3H9 transgenics: the representation of VK12/13 ranges from 64% for VH3H9 to 26% for VH3Germ line and 0% for VH56R (11, 57), whereas the representation of VK21D (VK21-4) is 78% for VH56R (11). We attribute the differences in editor usage in BALB/c transgenics to their efficiencies of editing H chains with different Arg content and location. Thus, VH56R can only be edited by VK21D or VK20, editors with the highest content of aspartate (11); but VH3H9 and VH3Germ line can be edited by these and other L chains.

**FIGURE 4.** Amino acid sequences of the Vk and VH genes of the Vk23-expressing IgG hybridomas derived from an H76R/MRL-lpr mouse. The Vk23 sequences (A) are compared with the germ line Vk23-43 and Vk23-45 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 Vk23 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).
H76R B cells from BALB/c mice have an unusual editor repertoire consisting of nearly all Vx38c (Table II). This is unexpected because H76R/Vx38c binds DNA (Table II). Why is Vx38c preferred as a 76R editor? Perhaps other editors are negatively selected for other reasons such as less than optimal heavy/light fit; alternatively, Vx38c may have a specificity that is positively selected. Indeed, H76R/Vx38c 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 Vx38c 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, Vx23 is the most frequent IgG-associated L chain. The shift in L chain usage is undoubtedly due to Ag selection, but the stage at which Vx23 arises could be central or peripheral. If the IgG Vx23 B cells came from rare Vx23-expressing IgM precursors that have escaped central tolerance, then they should show evidence of clonal expansion. However, the eight Vx23 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 Vx23 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 Vh than in VL (Fig. 4). Clone no. 123 is the extreme: its Vh has accumulated five mutations, and its Vk has none. Assuming that Vh and VL mutations are initiated at the same time during an immune response, this finding suggests that Vx23 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 (VH,D42). 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 Vg gene (VgRF) is repeatedly used by almost all the IgG anti-DNA Abs, indicating a strong Ag selection. The secondary rearrangement of the VgRF 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 VhD42 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 Vx23 in H76R/MRL-lpr mice is surprising because more variety of Vxs would be expected. Studies on VH3H9 and VH56R transgenics have shown that many L chains besides Vx23 can yield anti-dsDNAs in combination with the tg H chains (20, 25); however, the bias toward Vx23 cannot be attributed to expansion of one or a few clones (see above). Vx23 over-representation is common among anti-DNAAs in different autoimmune mice (33, 62–67). In contrast, Vx23 is one of the least frequently used Vg genes in non-autoimmune B cells: of 1040 Vg sequences compiled by Kabat et al. (35), only 12 (1%) are Vx23, and only four of these bound non-self Ags. Recently, Mohan and colleagues (68) compiled a L chain database grouping the Abs into
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 Vx23 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 (pI 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 to the B cell receptors. These edited B cells, perhaps by virtue of their 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.

Acknowledgments

We thank Drs. Tony Bakke for help with FACS analysis and Drs. Peter Ulrich and Agata Matejuk for critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References


