Breakdown of B Cell Tolerance in a Mouse Model of Systemic Lupus Erythematosus

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Summary

Anti-DNA antibodies, specifically those that stain nuclei in a homogenous nuclear (HN) fashion, are diagnostic of systemic lupus erythematosus (SLE) and the MRL-*lpr/lpr* SLE murine model. We have used a heavy chain transgene that increases the frequency of anti-HN antibodies to address whether their production in SLE is the consequence of a defect in B cell tolerance. Anti-HN B cells were undetectable in nonautoimmune-prone transgenic mice, but in MRL-*lpr/lpr* transgenic mice their Ig was evident in the sera and they were readily retrievable as hybridomas. We conclude that nonautoimmune animals actively delete anti-HN–specific B cells, and that MRL-*lpr/lpr* mice are defective in this process possibly because of the *lpr* defect in the *fas* gene.

SLE is a complex disease that has a spectrum of clinical manifestations thought to be the consequence of a dysfunctional immune system. Autoantibodies, specifically anti-DNA antibodies, are the serological hallmark of SLE (1). Although the etiology of serum autoantibodies is unknown, their presence has been attributed to a defect in the regulation of self-reactive B and/or T cells (2–6). Studies using self-reactive Ig transgenic (tg)[1] mice have demonstrated that B cells are normally subject to tolerance induction either by deletion or functional inactivation (7, 8). It is not clear, however, how the rules that have been established for B cell tolerance to exogenous antigens or neo-self antigens apply to antigens targeted in autoimmunity.

Anti-DNA antibodies from both SLE patients and animal models of SLE are heterogeneous in terms of their specificity for DNA and DNA/protein complexes (1, 9–12). Antibodies that stain nuclei and mitotic figures in a homogeneous fashion in the anti-nuclear antibody (ANA) assay are designated as anti-homogeneous nuclear (anti-HN). The presence of this subset of anti-DNA antibodies is one of the diagnostic criteria for SLE (13). Here we address the regulation of various anti-DNA antibodies in nonautoimmune mice and how this may be disrupted in autoimmune animals. Our approach has been to generate anti-DNA Ig tg mice and cross the anti-DNA Ig tg onto both nonautoimmune (BALB/c) and autoimmune (MRL-*lpr/lpr*) genetic backgrounds.

The MRL-*lpr/lpr* mouse provides a well characterized model of human SLE, developing anti-DNA antibodies and a lupuslike nephritis (10–12). The *lpr* mutation was recently shown to be the consequence of an insertion of a retrotransposon (ETn) into the second intron of the gene encoding the membrane antigen Fas (14–16). The resulting defect in mRNA splicing prevents production of normal levels of Fas protein. In mice homozygous for the *lpr* mutation (*lpr/lpr* mice) only 2–10% of Fas mRNA is wild type (15, 16), and Fas protein has not been detected in cell populations such as thymocytes and activated peripheral B and T cells which normally express Fas (17). The observation that Fas is a receptor whose engagement on certain transformed cell lines results in apoptosis (18) has led to the suggestion that a defect in apoptosis-mediated negative selection is the cause of autoimmunity in MRL-*lpr/lpr* mice (14). However, the involvement of Fas in selection of the immune repertoire is controversial. Several reports have indicated that MRL-*lpr/lpr* mice are not grossly defective in negative selection of T cells (19, 20) and are also capable of mediating appropriate positive selection (20), whereas other studies report defects in the induction and maintenance of T cell tolerance (21, 22). Whether the *lpr* mutation has a direct effect on B cell repertoire formation in MRL-*lpr/lpr* mice is not known. However, since the *lpr* mutation when crossed to a number of mouse strains results in the induction of various autoantibodies (including anti-DNA antibodies) (23), the *lpr* mutation appears to have a direct role in the genesis of autoantibodies.

We have used an Ig H chain tg that increases the frequency of anti-DNA autoantibodies to address whether the production of anti-DNA antibodies in MRL-*lpr/lpr* mice is the consequence of a breakdown of B cell tolerance. The design of our Ig tg was based on the observation that a particular H chain, VH3H9, has been recurrently isolated from anti-DNA antibodies arising spontaneously in MRL-*lpr/lpr* mice, indicating that this VH has inherent DNA-binding capacity (24).

[1] *Abbreviations used in this paper:* ANA, anti-nuclear antibody; anti-HN, anti-homogenous nuclear; tg, transgenic.

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Moreover, this VH can pair with many different VL genes to give anti-DNA antibodies as well as non-DNA binding antibodies (25, and this report). By crossing the VH3H9 tg onto BALB/c and MRL-*lpr/lpr* genetic backgrounds and assessing the specificity of their peripheral B cell repertoires, we have identified the kinds of anti-DNA antibodies that are present in normal mice and the kinds that are only found in the autoimmune-prone animals. We found that anti-HN B cells were undetectable in nonautoimmune-prone VH3H9 tg mice, whereas they were readily retrievable as hybridomas and their Ig was evident in the sera of MRL-*lpr/lpr* VH3H9 tg mice. This suggests that nonautoimmune animals actively delete anti-HN–specific B cells, and that MRL-*lpr/lpr* mice are defective in this process.

Materials and Methods

Mice. The generation of VH3H9 tg mice using a construct consisting of the MRL-*lpr/lpr*–derived 3H9 V region combined with the BALB/c μ constant region has been described previously (26). The tg has been backcrossed onto the nonautoimmune BALB/c and the SLE-prone MRL-*lpr/lpr* backgrounds: homozygosity for the *lpr* mutation in *fas* was determined for mice on the MRL background by scoring for development of lymphadenopathy and the presence of ($CD4^-$, $CD8^-$, $CD3^+$) double negative T cells that characterize *lpr/lpr* mice (12). In addition, the earliest backcross mouse (MRL1 at backcross 2) was verified as *lpr/lpr* by PCR amplification which distinguishes the *lpr* allele from the wild-type allele (data not shown). Each VH3H9 tg mouse is at least backcross 2, which corresponds to three matings onto and ~88% of a given genetic background. Mice carrying the VH3H9 tg were identified by amplification of tail DNA using the VH3H9 tg specific primers (26): VH3H9 Leader 5'CTGTCAGGAACTGCAGGTAAGG3'; VH3H9CDR3 5'CATAACATAGGAATATTTACTCCTCGC-3'. Non-tg MRL-*lpr/lpr* mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and BALB/cJ mice were purchased from Charles River Laboratories (Wilmington, MA).

B Cell Hybridoma Production. Spleen cells from mice were fused without further manipulation to the Ig^- myeloma Sp2/0 (27). Cells were plated at limiting dilutions and only wells bearing single colonies were expanded for analysis.

ELISA Assays. Isotype and Ig concentration were determined using indirect solid-phase ELISA assays, as described previously (26). Isotype was determined using anti-κ or anti-λ L chain antibodies (Southern Biotechnology Associates, Birmingham, AL) as the primary antibody, then developing with alkaline phosphatase–labeled anti-IgM or anti-(IgG1+IgG2a+IgG2b+IgG3+IgA) antibodies (Southern Biotechnology Associates). Ig concentration was determined by comparing samples to a titrated isotype-matched standard. Binding to ssDNA was detected in the same manner, except that boiled and snap-chilled sonicated salmon sperm DNA (Sigma Chemical Co., St. Louis, MO) at 14 μg/well in PBS was used in place of primary antibodies, as described previously (26). Values of five times the negative control were considered positive.

Preparation and Analysis of DNA. DNA was prepared from confluent hybridoma cell cultures using proteinase K digestion followed by phenol extraction. 10 μg of DNA was then digested with either EcoRI or BamHI, size separated, and blotted onto Zeta-probe blotting membrane (Bio-Rad, Richmond, CA). DNA rearrangements at the H chain locus were detected by probing EcoRI membranes with pJ11 (28), and at the L chain locus by probing BamHI membranes with pECκ (29).

Anti-HN Antibody Detection. The presence of anti-HN antibody in sera and hybridoma supernatants was detected using permeabilized HEP-2 cells as the substrate (Antibodies Incorporated, Davis, CA). The manufacturer's instructions were followed except for substitution of undiluted supernatants when hybridomas were assayed.

Sequence Analysis of Ig H and L Chain mRNA. Cytoplasmic RNA was isolated from ~10^5 hybridoma cells by NP-40 lysis followed by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. Constant region–specific oligonucleotide primers were used to direct synthesis of cDNA copies of the H and L chain V regions, which were then amplified in the PCR using the same constant region primers and degenerate primers that hybridize to the 5'-ends of H and L chain V regions as described previously (30), except that unfractionated cDNA products were subjected to amplification. The dsDNA copies of the H and L chain V regions were fractionated by agarose gel electrophoresis, isolated using GeneClean (Bio101, distributed by American Bioanalytical, Natick, MA), and directly sequenced using Sequenase (USB, Cleveland, OH) and either the constant or V region–specific primer that was used in the amplification reaction to direct synthesis. Sequence was read and translated using the Wisconsin program (31). Sequence comparison was carried out by searching the EMBL/GenBank/DDBJ databases and Kabat et al. (32).

Primers. Production of cDNA was carried out using a 3' primer of interest (e.g., C κ for κ L chains). Amplification utilized 3' and 5' primer pairs as indicated for a given chain. Sequencing was carried out using either the 3' or 5' primer, and sequence reverse complimented as appropriate. Primer pairs used were the following: κ (3') C κ: 5'GTTGGTGCAGCATCAGC 3', (5') L5': 5'CCAGTTCCGAGCTCCAGATGACCCAGACTCCA3'; λ1 (3') Cλ1: 5'CTTCAGAGGAAGGTGGAAACAGGGTG3', (5') λ1L: 5'TCTCCTGGCTCTCAGCTCAG3'; λ X (3') Cλ2-3: 5'GGTGAG(A/T)GTGGGAGTGGACTTGGGC3', (5') VλX: 5'GAGCTTAAGAAAGATGGAAGCCA3', μ (3') IgM: 5'AGACATTTGGGAAGGAC3', (5') VH5'1: 5'GAGGTGAAGCTGGTGGAG(T/A)C(T/A)GG3'; and γ (3') Cγcross: 5'GGGGCCAGTGGATAGAC3', (5') VH5'1: 5'GAGGTGAAGCTGGTGGAG(T/A)C(T/A)GG3'.

Primers described previously were the following: L5' was adapted from L4/L5 (33); λ 1 primers were derived from the germline λ1 leader and Cλ1 sequence reported in (34); similarly the Cλ2-3 primer was derived from sequence reported in (35); the VλX primer was reported by (36); the IgM and Cγcross primers were reported in (37); whereas the VH5'1 primer was taken from (38). To verify the use of the VH3H9 tg with the γ constant region, cDNA was made using Cγcross, then a nested primer specific for CDR3 of VH3H9 was used with VH5'1 to amplify. The VH3H9 CDR3 primer is the same used in genotyping mice.

Results and Discussion

The VH3H9 tg paired with endogenous L chains generates a range of anti-DNA and non-DNA reactive antibodies. We have used this tg as a means of following the fate of anti-DNA B cells in nonautoimmune and autoimmune-prone mice to address whether the production of anti-DNA antibodies in SLE is the consequence of a breakdown in B cell tolerance. The tg has been backcrossed onto the nonautoimmune BALB/c and the SLE-prone MRL-*lpr/lpr* backgrounds for the number of generations indicated in Table 1 and mice are referred to as "BALB/c" and "MRL-*lpr/lpr*" to indicate the predominant genetic background. The *fas* gene in the BALB/c background is wild type (+/+), whereas all the MRL- *lpr/lpr* mice were

Table 1. *Profiles of Mice Used To Generate B Cell Hybridomas*

	Mouse	Age	Sex	Backcross	*fas*	Serum specificity	
						ssDNA	ANA
		wk					
BALB/c VH3H9 tg							
	BALB1	37	M	2	+/+	–	–
	BALB2	16	M	3	+/+	–	–
MRL-*lpr/lpr* VH3H9 tg							
	MRL1	13	M	2	*lpr/lpr*	+	HN
	MRL2	14	F	3	*lpr/lpr*	+	HN
	MRL3	16	F	5	*lpr/lpr*	+	HN
MRL-*lpr/lpr* non-tg							
	#2	24	F	NA	*lpr/lpr*	+	HN
	#22	20	F	NA	*lpr/lpr*	+	HN
	#31	36	M	NA	*lpr/lpr*	+	HN
	#34	14	F	NA	*lpr/lpr*	+	HN
	#40	12	F	NA	*lpr/lpr*	+	HN
	#51	16	M	NA	*lpr/lpr*	+	HN
	#108	16	M	NA	*lpr/lpr*	+	HN

Profiles of the VH3H9 tg mice and non-tg MRL-*lpr/lpr* mice used to generate B cell hybridomas are presented. Mice are either homozygous for the wild-type *fas* gene (+/+), or the mutated *fas* gene (*lpr/lpr*). Serum specificity for each mouse was determined using an ELISA assay for ssDNA binding and the HEP-2 immunofluoresence assay for detection of anti-HN antibodies as described in Fig. 1. The BALB2 mouse has been previously reported (25).

selected for homozygosity of the *lpr* mutation. Each VH3H9 tg mouse is at least backcross 2 which corresponds to having ~88% of a given genetic background. Features of the mice used in this study are listed in Table 1.

Specificity of VH3H9 B Cells in BALB/c and MRL-lpr/lpr Mice. The VH3H9 tg can pair with different L chains from the endogenous pool to produce three different specificities: non-DNA Igs, anti-ssDNA Igs, and anti-HN Igs (25, 26). To assess how BALB/c and MRL-*lpr/lpr* mice regulate the production of different kinds of antibodies in their serum, individual mice were examined for the ability of their serum to bind ssDNA by ELISA, and to display anti-HN ANAs. Anti-HN Igs are a reliable indicator of SLE that distinguishes the sera of MRL-*lpr/lpr* mice from those of nonautoimmune strains (10, 39). In addition, the anti-HN subset of anti-DNA antibodies has been implicated in renal pathology (39). As was previously described, the serum from BALB/c VH3H9 tg mice showed undetectable binding to ssDNA and lacked anti-HN Ig (26, and this report). In contrast, 24 of 27 MRL-*lpr/lpr* VH3H9 mice contain anti-ssDNA and anti-HN serum Igs, three of which are described in Table 1. A representative example of the HN staining pattern from an MRL-*lpr/lpr* hybridoma is presented in Fig. 1.

To see whether MRL-*lpr/lpr* VH3H9 tg mice have higher titers of serum anti-HN antibodies than non-tg MRL-*lpr/lpr* mice, anti-HN titers were determined for 9 MRL-*lpr/lpr* VH3H9 tg and 10 age-matched MRL-*lpr/lpr* non-tg mice. All of these mice were previously typed as seropositive for anti-HN antibodies. As shown in Fig. 2, the total anti-HN Ig titers from the VH3H9 tg and non-tg MRL-*lpr/lpr* mice overlap. The tg, however, does dramatically increase the titers of anti-HN IgM: all of the MRL-*lpr/lpr* VH3H9 tg mice had serum titers of anti-HN IgM which approximate that of their anti-HN Ig titers whereas only three of the MRL-*lpr/lpr* non-tg mice were seropositive for anti-HN IgM. Most of the anti-HN Ig in the VH3H9 tg MRL-*lpr/lpr* mice is IgM which is likely due to the constraints of the IgM VH3H9 tg. The presence of the VH3H9 tg increased the frequency of anti-HN B cells in the MRL-*lpr/lpr* VH3H9 tg mice (see below) yet this is not reflected by an increase in anti-HN Ig in the serum, suggesting that other factors dictate serum expression. The kinetics of anti-HN Ig serum expression is the subject of another study.

To analyze the B cell repertoires of these mice in greater detail, B cell hybridomas were generated from spleen cells from each of these VH3H9 tg mice (i.e., two BALB/c VH3H9 tg and three MRL-*lpr/lpr* VH3H9 tg mice) as well as from seven MRL-*lpr/lpr* mice that do not bear the VH3H9 tg. The MRL-*lpr/lpr*–derived hybridoma panels were all generated with unmanipulated spleen cells. The BALB1 spleen cells were likewise not activated before fusion to facilitate comparison to a large set of hybridomas that had been derived from spontaneous B cell fusions in various SLE-prone mice (for example, 24, 40, 41). The BALB2 VH3H9 tg hybridoma

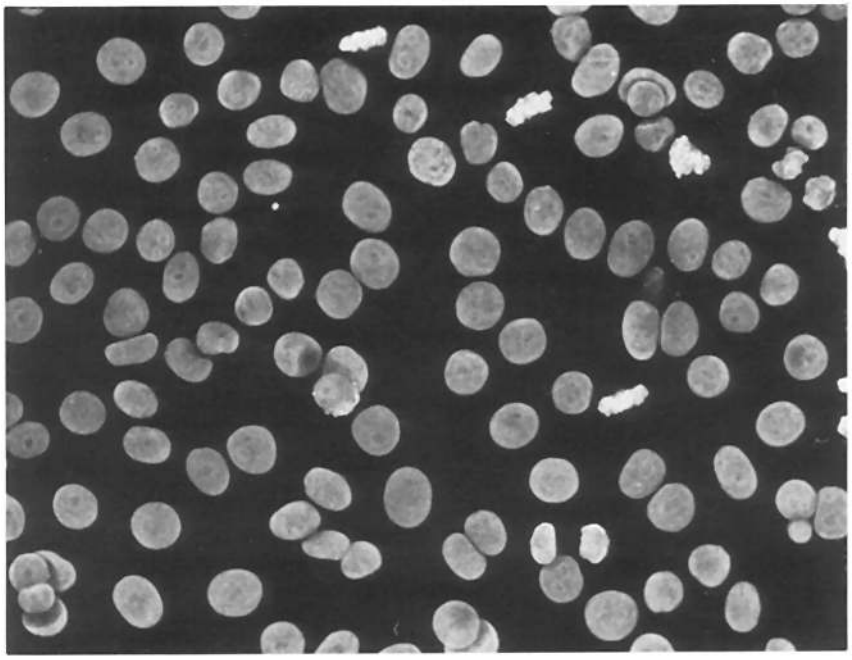

Figure 1. Anti-HN antibodies stain the nuclei of HEP-2 cells in a homogenous fashion. The MRL1-17 hybridoma supernatant was incubated with permeabilized HEP-2 cells and staining detected with goat anti-mouse fluoresceinated secondary antibodies.

panel was generated using spleen cells that had been activated in vivo with LPS before fusion, as was described previously (25). All of the hybridomas that were retrieved from the BALB/c VH3H9 tg mice were of independent origin as determined by Southern blot analysis using the rearrangement patterns of both the Ig H and L chain loci (data not shown). Among the MRL-*lpr/lpr* VH3H9 tg hybridomas, however,

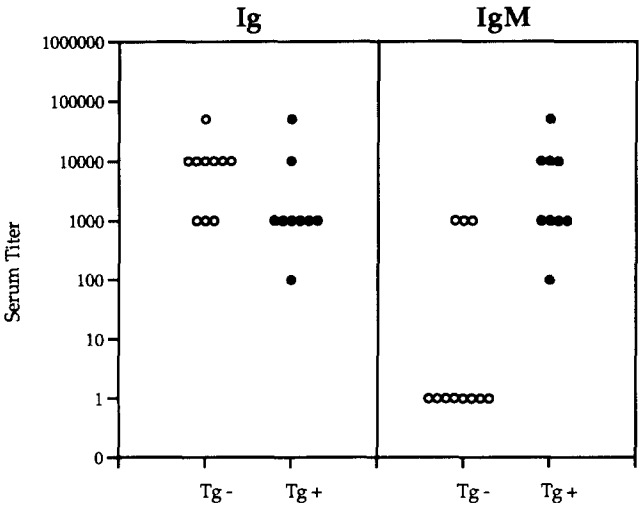

Figure 2. Serum titers of anti-HN ANAs from MRL- *lpr/lpr* non-tg (*Tg*$^-$) and VH3H9 tg (*Tg*$^+$) mice. Sera from 10 MRL-*lpr/lpr* non-tg mice and 9 MRL-*lpr/lpr* VH3H9 tg mice which were seropositive for anti-HN ANAs were diluted in PBS/Azide and assayed for anti-HN titers by immunofluorescence. ANA patterns were visualized with either goat anti-mouse Ig-FITC or goat anti-mouse IgM-FITC. The serum titer is defined as the reciprocal of the last dilution at which the anti-HN staining pattern is discernible. All mice were 15–30 wk of age, and had been backcrossed for at least seven generations onto the MRL- *lpr/lpr* background.

a total of three groups of clonally expanded B cells were retrieved, two from MRL1 and one from MRL3 (Roark, J., C. L. Kuntz, K.-A. Nguyen, L. Mandik, M. Cattermole, and J. Erikson, manuscript submitted for publication).

All Ig$^+$ hybridomas were analyzed by ELISA for binding to ssDNA and by immunofluorescence for the presence of anti-HN Ig (Table 2). The most striking difference between the BALB/c and the MRL-*lpr/lpr*–derived hybridoma panels is that anti-HN hybridomas were recovered from the MRL-*lpr/lpr* VH3H9 tg mice, but not from the BALB/c VH3H9 tg mice (Table 2). Anti-ssDNA hybridomas, on the other hand, were recovered from both sets of mice. It is noteworthy that the VH3H9 tg greatly augmented the frequency of both anti-ssDNA and anti-HN hybridomas in the MRL-*lpr/lpr* background as compared with MRL-*lpr/lpr* tg(−) mice. Specificity for ssDNA was also increased among hybridomas from the BALB/c VH3H9 tg mouse as compared with frequencies reported for BALB/c tg(−) mice (42, 43). The increased frequency of these specificities is consistent with previous observations (25, 26) that the VH3H9 tg paired with a variety of endogenous L chain genes give antibodies that can bind to DNA.

Together, these specificity data support previous conclusions that normal mice actively regulate anti-DNA antibodies (18, 26, 44). The manifestations of this regulation in BALB/c VH3H9 tg mice appear to vary for ssDNA- versus HN-specific B cells. Anti-ssDNA B cells dominate the B cell repertoire of BALB/c VH3H9 tg mice, yet the tg mice do not have serum anti-DNA antibodies (26). As described previously (26), this suggests a functional block in their ability to differentiate into antibody-secreting cells. Anti-HN B cells, however, were not rescued in hybridoma panels nor was their Ig present in the sera, suggesting that they are deleted from

Table 2. *Specificity of Hybridoma Panels*

	Total No.	αssDNA	αHN
		%	
BALB/c VH3H9 tg			
BALB1	51	33	0
BALB2	49	39	0
MRL-*lpr/lpr* VH3H9 tg			
MRL1	167	76	39
MRL2	90	53	13
MRL3	13	69	46
MRL-*lpr/lpr* non-tg			
#2	61	2	2
#22	25	0	0
#31	50	2	0
#34	96	18	0
#40	35	6	3
#51	23	4	0
#108	331	6	1

The specificity was determined for hybridomas in each panel using an ELISA assay for ssDNA binding and the HEP-2 immunofluoresence assay for detection of anti-HN antibodies as described in Fig. 1. Specificity of the total hybridoma panels for the VH3H9 tg mice and a panel of non-tg MRL-*lpr/lpr* mice is presented. The BALB2 mouse has been previously reported (25). Both the BALB1- and BALB2-derived hybridomas have the same specificity profile as a third VH3H9 tg hybridoma panel from a nonautoimmune background described previously (26).

the B cell repertoires. Additional sets of Ig trangenics support this conclusion: no B cells with the transgene specificity were detected in the periphery in two examples where the pairing of the H and L chain transgenes generates anti-HN Igs in vitro: VH3H9/Vκ4 and VH3H9 56R/Vκ8 (45, 46).

In the autoimmune-prone MRL-*lpr/lpr* VH3H9 tg genetic background, anti-HN hybridomas were recovered and serum anti-HN Igs were present (Tables 1 and 2). In view of the clear regulation of anti-DNA B cells from BALB/c VH3H9 tg mice, this suggests that there is a defect in the regulation of anti-HN B cells in the MRL-*lpr/lpr* background. Anti-ssDNA B cells were also recovered from MRL-*lpr/lpr* VH3H9 tg mice, as they were from BALB/c VH3H9 tg mice. Unlike what was observed in the BALB/c background, however, serum anti-ssDNA Igs were detectable in the MRL-*lpr/lpr* mice. It is unclear whether the anti-HN and anti-ssDNA Igs represent different sets of antibodies: we have found using monoclonal antibodies that anti-HN Igs are often also positive in the anti-ssDNA assay, whereas there are anti-ssDNA Igs that are non-HN. Because the serum expression of anti-HN Igs also renders these mice seropositive for anti-ssDNA, the experiments described here do not address the fate of anti-ssDNA (non-HN) B cells in the MRL-*lpr/lpr* background. They do clearly show, however, that the MRL-*lpr/lpr* mice are defective in the negative regulation of anti-HN B cells.

Genetic Basis for Anti-DNA Antibodies in VH3H9 tg Mice. To address the genetic basis for the presence of anti-HN hybridomas in the MRL-*lpr/lpr* VH3H9 tg panels and their absence in the BALB/c VH3H9 tg panels, hybridomas were analyzed for the sequences of their H and L chain Ig V region mRNAs. Sequence analysis of the LPS-activated hybridomas has been previously described and is not presented here (25). All of the hybridomas from the BALB1 and MRL3 fusions were analyzed. A subset of hybridomas from the larger MRL1 and MRL2 panels were sequenced, with an emphasis on those that gave anti-HN reactivity. A fraction of hybridomas from both BALB/c and MRL-*lpr/lpr* mice were found to express mixed L chain sequences, most likely due to the expression of out-of-frame transcripts that were amplified in the PCR reactions used for sequencing. In addition, whereas the BALB/c VH3H9 tg–derived hybridomas all exclusively expressed an unmodified VH3H9 tg-encoded H chain, approximately half of the MRL-*lpr/lpr* VH3H9 tg hybridomas coexpressed an additional H chain mRNA (data not shown). The hybridomas that coexpressed an endogenous H chain did not account for the HN specificity in the MRL-*lpr/lpr* hybridoma panels, since subcloning experiments that segregate the endogenous VH from the VH3H9 tg have shown that the HN specificity associates with the VH3H9 tg (Roark, J. H., et al., manuscript submitted) and 61% of the anti-HN B cells recovered exclusively expressed the VH3H9 tg H chain. To definitively assign specificity to gene segment usage, we have confined our discussion of the sequence analysis to those hybridomas that only expressed one H chain gene and only one κ and/or λ L chain. Table 3 shows the hybridomas that fit these criteria.

Substantial Overlap in V κ Gene Segment Use Between BALB/c and MRL-lpr/lpr Mice. L chain V and J gene sequences and specificity for κ-expressing hybridomas are presented in Fig. 3 *A*. All of these hybridomas express the VH3H9 tg H chain exclusively (data not shown). There is considerable overlap in V κ gene usage in hybridomas from BALB/c and MRL-*lpr/lpr* VH3H9 tg mice. The most striking example of this is the Vκ12a gene: 54% of BALB1 VH3H9 tg and 14% of

Table 3. *Specificity of Sequenced VH3H9 tg Hybridomas*

	No. of hybridomas	αssDNA	αHN
		%	
BALB/c VH3H9 tg			
BALB1	38	26	0
MRL-*lpr/lpr* VH3H9 tg			
MRL1	11	100	91
MLR2	20	70	30
MRL3	5	80	60

The specificity of the hybridomas that express the VH3H9 tg and a single VL (or in two cases the VH3H9 tg with a κ and a λ VL) are presented.

A. V kappa amino acid sequences from VH3H9 tg hybridomas

V Kappa 1		FW1	CDR1	FW2	CDR2	FW3	CDR3	FW4	JL	ssDNA	HN
A	BALB1-1		ASISC KSTKSLLNSDGFTYLD	WYLQKPGQSPQLLIY	LVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FSQNYLPLT	FGGG	1	-	-
A	BALB1-12		----------	----------	-------	----------	---------	F-A-	5	-	-
A	BALB1-22		----------G	----------	-------	----------	---------	F---	1	-	-
A	BALB1-85	SLPVNIGDQ----	----------G	----------	-------	----------	-------F-	F-T-	4'	-	-
V Kappa 2											
A	BALB1-35		SISC KSSQSLLDSDGKTYLN	WLLQRPGQSPKRLIY	LVSKLDS	GVPDRFTGSGSGTDFTLKISRVEAEDLGVYYC	WQGTHFPLT	FGAG	5	+	-
A	MRL2-41		----------	----------	-------	----------	-------R-	--G-	1	+	-
A	MRL2-51*		--	----------	-------	----------	-------Y-	--S-	2'	+	-
V Kappa 4/5											
B	BALB1-26	SASPGEKVTMIC	RASSSVSSSYLH	WYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGSG	4	+	-
C	BALB1-19	-----	S------YMY	-----P--R-L--D-	-------	----------RM----------	--W-S--PMLT	--A-	5	+	-
E	BALB1-32	--I--	S-T----YMH	-F---P-T-------	-------	----------RM----------	--R-S--F-	----	4	+	-
F	BALB1-53	----Q---I--	S-----NYMH	-----L--------	D--K--P	----------M------S-F-	H-W-S--P-	--A-	5	+	-
F	BALB1-54	AIM-----Q---I--	S-----NYMH	-----L--------	D--K--P	----------M------S-F-	H-W-S--P-	--T-	4'	+	-
F	MRL2-186	1--	S-----NYMH	-----L--------	D--K--P	----------M------S-F-	H-W-S----	--A-	5	+	-
G	MRL1-29	----------R-----	S----------	-----P--------	-------	----------M--------C-	--------K	--A-	5	+	-
G	MRL1-46	-	S-------N---	-----P--------	-------	----------M---------	L------Y-	----	2'	+	+
G	MRL1-50	-	S------A----	-----P--------	-------	---D--------M---------	-------Y-	----	2'	+	+
G	MRL1-56		----	-----P--------	-------	----------M---------	-------S-	----	2'	+	+
V Kappa 9											
A	BALB1-67		ASQGISSNIG	WLQQKPGKSFKGLIY	HGTNLED	GVPSRFSGSRSGADYSLTISSLESEDFADYYC	VQYAQFPPT	FGGG	1	-	-
C	MRL2-17		VSLTC R---D-GNSIN	----E-DGNI-R---	ATSR-DF	D--E--------S----------V----	L--GS--L-	--A-	5	+	+
C	MRL2-169		- ----D-G-SLN	----E-DGTI-R---	ATSR-DS	---E--------S----------V----	Q--GS--L-	--A-	5	+	+
D	MRL2-117	SSLSASLGER-----	----E--GYLS	------DGTI-R---	AAST-DS	---K--------S----------	L---SY-Y-	--S-	2'	+	-
V Kappa 12/13											
A	BALB1-3		TVTITC RASENIYSNLA	WYQQKQGKSPQLLVY	AATNLAD	GVPSRFSGSGSGTQYSLKINSLQSEDFGSYYC	QHFWGTPPT	FGSG	2'	-	-
A	BALB1-6		------ ----------	----------	-------	----------	-------Y-	--G-	2	-	-
A	BALB1-13		------ ----------	----------	-------	----------	-------W-	--G-	1	-	-
A	BALB1-17		------ ----------	----------	-------	----------	---------	--A-	5	-	-
A	BALB1-36		------ ----------	----------	-------	----------	-------Y-	--G-	2	-	-
A	BALB1-38	GE------	----------	----------	-------	----------	---------	--G-	1	-	-
A	BALB1-39		---	----------	-------	----------	-------Y-	----	2'	-	-
A	BALB1-45	LSVSV--------	----------	----------	-------	----------	-------Y-	----	2'	-	-
A	BALB1-52		---	----------	-------	----------	-------Y-	----	2'	-	-
A	BALB1-57	AS----------	----------	----------	-------	----------	---------	--A-	5	-	-
A	BALB1-59	----------	----------	----------	-------	----------	-------Y-	----	2'	-	-
A	BALB1-60	----------	----------	----------	-------	----------	-------Y-	--G-	2	-	-
A	BALB1-68	----------	----------	----------	-------	----------	-------Y-	----	2'	-	-
A	BALB1-71	----------	----------	----------	-------	----------	-------Y-	--G-	2	-	-
A	BALB1-72*	----------	----------	----------	-------	----------	-------Y-	--G-	2	-	-
A	BALB1-77	----------	----------	----------	-------	----------	---------	--A-	5	-	-
A	BALB1-86	----------	----------	----------	-------	----------	-------Y-	--G-	2	-	-
A	BALB1-88			----------	-------	----------	-------Y-	-	2?	-	-
A	MRL2-14	----------	----------	----------	-------	----------	-------Y-	----	2'	-	-
A	MRL2-18		----------	----------	-------	----------	-------L-	--A-	5	-	-
A	MRL2-75		----------	----------	-------	----------	---------	----	2'	-	-
A	MRL2-146	-	----------	----------	-------	----------	-------W-	--G-	1	-	-
A	MRL2-162		----------	----------	-------	----------	-------Y-	----	2'	-	-
C	BALB1-8	----------	----------	----------	------	----------F--------P--------	-------W-	--G-	1	+	-
D	BALB1-62	----A---------	--------Y--	----------	N-KT--E	----------F--------P--------	--HY------	--G-	1	+	-
D	MRL2-154	----A---------	--------Y--	----------	N-KT--E	----------F--------P--------	--HY---Y-	----	2'	+	+
D	MRL3-14	-A---------	--------Y--	----------	N-KT--E	----------F--------P--------	--HY---W-	--G-	1	+	-
D	MRL3-34	-A---------	--------Y--	----------	N-KT--E	----------F--------P--------	--HY---Y-	----	2'	+	+
D	MRL3-41	-A---------	--------Y--	----------	N-KT--E	----------F--------P--------	--HY---Y-	----	2'	+	+
E	MRL1-20	----------	G-----GA-N	----------	------I-	G------- -I---------R-Y----S--H-D-VAT---	-NVLS--Y-	----	2'	+	+
V Kappa 19/28											
C	MRL2-111	KFMSTSVGDRVSISC	KASQNVGNIIA	WYQQKPGQSPKALIY	LASYRYS	GVPDRFTGSGSGTDFTLTISNVQSEDLAEYFC	QQYSSSPYT	FGSG	2'	+	-
E	MRL1-26	-----E----T-	------TNV-	-----A---LEL---	G--N-HT	----------T------MIN---	E----Y---	----	2'	+	+
V Kappa 21											
D	BALB1-9	SLAVSLGQRATISC	KASQSVDYDGDSYMN	WYQQKPGQPPKLLIY	AASNLES	GIPARFSGSGSGTDFTLNIHPVEEEDAATYYC	QQSPEDPWT	FGGG	1	-	-
D	BALB1-11		----------	----------	-------	----------	L------Y-	--S-	2'	-	-
D	BALB1-51		---------	----------	-------	----------	-------P-	--S-	2'	-	-
V Kappa 23											
A	MRL1-17		AC RASQSISNYNH	WYQQKSHESPRLLIK	YASQSIS	GIPSRFSGSGSGTDFTLSINSVETEDFGMYFC	QQSNSWPHT	FGSG	2'	+	+
A	MRL2-171	SVTPGDRVSLS-	-------K---	----------	------	----------	---K---L-	--A-	5	+	+
V Kappa 32											
A	BALB1-7		TIRC ITSTDIDDDMN	WYQQKPGEPPKLLIS	EGNTLRP	GVPSRFSSSGYGTDFVFTIENTLSEDVADYYC	LQSDNMPYT	FGSG	2'	-	-
A	MRL2-57†		KV---- ----------	----------N----	---A---	----------	--N------	----	2'	-	-
V Kappa 33/34											
A	BALB1-25		SVSLGDRVTITC KASEHINSWLA	WYQQKPGNAPRLLIS	GATSLET	GVPSRFSGSASGKDYTLSITSLQTEDVATYYC	QQYWGTPPT	FGGG	1	-	-
A	MRL3-55		---------- ----------	----------	-------	----------	-------Y-	--S-	2'	-	-
V Kappa 38											
A	BALB1-5		KVTITC KASQDINKYIA	WYQHKPGKGPRLLIH	YTSTLQP	GIPSRFSGSGSGRDYSFSISNLEPEDIATYYC	LQYDNL.YT	FGGG	2	+	-
A	BALB1-70		SASLGG------ ------K-N--	----------W	-------	----------	------PW		?	-	-

B. V lambda amino acid sequences from VH3H9 tg hybridomas

Lambda 1											
	BALB1-72*	TQDSALTTSPGETVTLTC	RSSTGAVTTSNYAN	WVQEKPDHLFTGLIG	GTNNRAP	GVPARFSGSLIGDKAALTITGAQTEDEAIYFC	ALWYSNHWV	FGGG	1	-	-
	MRL1-45	----------	----------	----------	-------	----------	---------	----	1	+	+
	MRL1-70	----------	----------	----------	-------	----------	---------	----	1	+	+
	MRL1-166	----------	----------	----------	-------	----------	------L--	----	1	+	+
	MRL1-333	----------	----------	----------	-------	----------	---------	----	1	+	+
	MRL2-51*	----------	----------	----------	-------	----------	---------	----	1	+	-
	MRL2-63	----------	----------	----------	-------	----------	---------	----	1	+	+
	MRL2-98	----------	----------	----------	-------	----------	---------	----	1	+	+
	MRL2-120	----------	----------	----------	-------	----------	---------	----	1	+	+
	MRL3-21	----------	----------	----------	-------	----------	---------	----	1	+	+
Lambda X											
	MRL2-20				LKKDGSHSTGD	GIPDRFSGSSSGADRYLSISNIQPEDEAIYIC	TIKEQFVYV	FGGG	2	-	-

Figure 3. Correlation of Ig gene segment use with specificity. VH3H9 tg antibodies were sequenced for L chain VJ and H chain VDJ genes and their specificity determined by ELISA for ssDNA binding and by immunofluorescence for HN staining. Only hybridomas that expressed the VH3H9 tg alone with a single L chain gene are presented, unless otherwise indicated. κ L chain sequences (*A*) and λ L chain sequences (*B*) are presented.

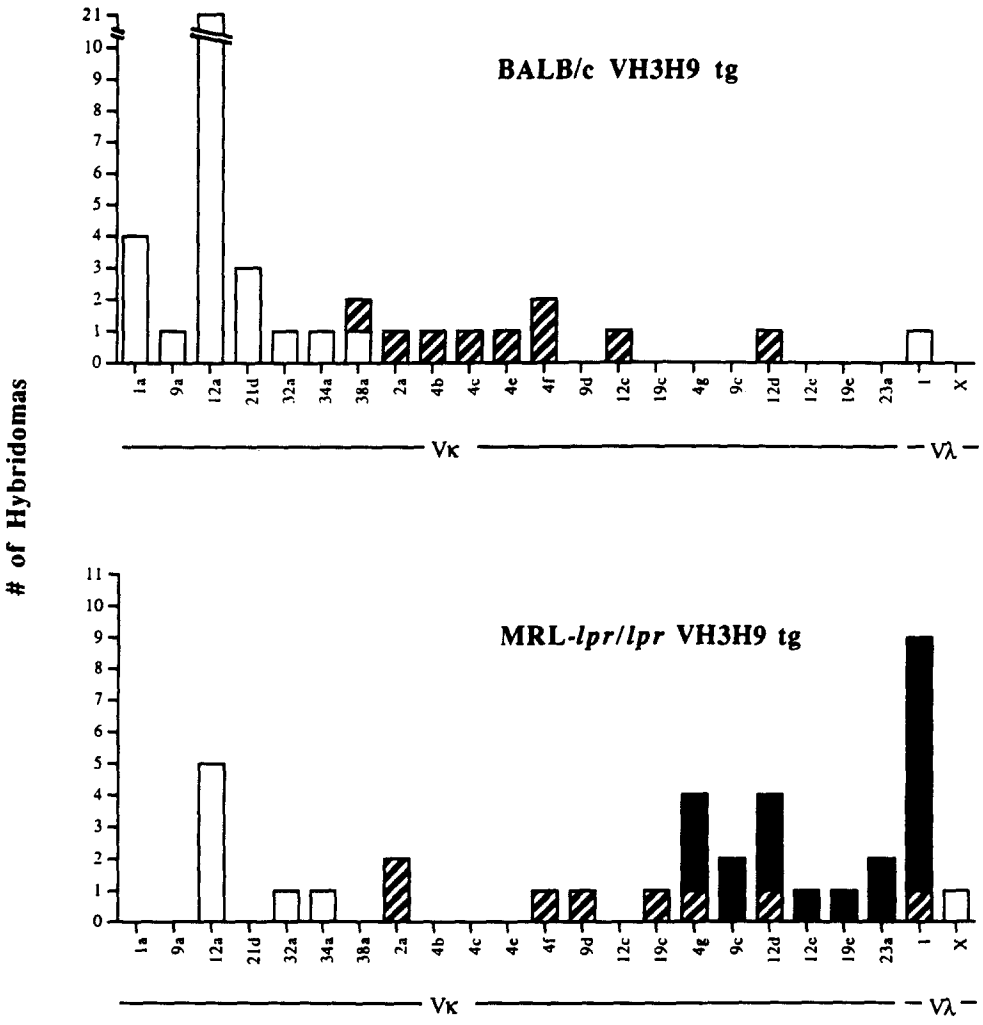

Figure 4. Summary of endogenous V κ and V λ gene use by VH3H9 tg hybridomas. Gene designations and specificity were determined as described in the legend to Fig. 3. L chain genes which pair with the VH3H9 tg to generate non-DNA (*open boxes*), anti-ssDNA (*hatched boxes*), or anti-HN (*filled boxes*) Igs are compared. (*Top*) Summarizes the V genes used by the BALB/c VH3H9 tg-derived hybridomas; (*bottom*) summarizes the V genes used by MRL-*lpr/lpr* VH3H9 tg-derived hybridomas expressing the VH3H9 tg with a single L chain gene, except for the κ/λ coexpressing hybridomas BALB-72 and MRL2-51. BALB-72 and MRL2-51 are therefore included in both the appropriate κ and λ columns.

the MRL-*lpr/lpr* VH3H9 tg sequences use the same Vκ12a gene. The frequency of Vκ12a in the MRL-*lpr/lpr* VH3H9 tg hybridomas may actually be an underestimate of its representation in the total hybridoma panel since we biased our sequence analysis toward anti-HN hybridomas. The overrepresentation of Vκ12a was also evident in the LPS-induced BALB/c VH3H9 tg hybridoma panels (25). Why this gene segment is overrepresented is unclear. Reports of overrepresentation of L chain gene segments include the observation that there is an increased frequency of Vκ4 family rearrangements in pre-B cell lines (47), and that particular Vκ4 members within the Vκ4 family are preferentially employed (48). The Vκ12a overrepresentation we observe may be the consequence of specificity-independent mechanisms such as the frequency of rearrangements of this particular L chain gene, or the consequence of specificity-dependent (positive) selection in associ-

A sequence was designated the same gene and given the same lowercase letter suffix if there were seven or fewer nucleotide differences (97.5% nucleotide similarity), with no more than four of these in framework regions. Although an arbitrary definition, for the most part sequences either fell into this definition or were quite clearly outside it. Framework (*FW*) and complementarity determining regions (*CDRs*) are shown above the sequences and separated by a space. The Vκ12d sequences discussed in the text are boxed. Gene families were assigned based upon homology to published sequences (32). The lowercase letter designation is used to distinguish among individual genes (e.g., Vκ12a), and are the same as those used previously in the BALB/c VH3H9 tg mice, where appropriate (25). Several sequences which were observed in the previous report of BALB/c VH3H9 tg mice (25) were not observed in the current study, such as Vκ4a. Jκs are assigned to the known BALB/c Jκ germline gene segments (55). MRL-*lpr/lpr* mice have the Jκ2′ allelic variant of Jκ2 which has a serine at position 100 and a methionine at position 106 compared with the glycine and isoleucine of Jκ2. Note that Jκ2 and Jκ2′ occupy the same chromosomal position relative to the other Jκs (56). The backcross 2 BALB/c VH3H9 tg mouse used in this study carries both the BALB/c Jκ2 locus and a J locus containing the same allelic variant found in the MRL- *lpr/lpr* mice which was most likely contributed by the SJL allele of the (C57Bl×SJL)F1 VH3H9 tg founder (56). In the case of BALB-70 and MRL1-219, insufficient sequence was obtained for assignment of the J gene used, so ND (not determined) is listed. (*) MRL2-51 and BALB-72 coexpress κ and λ L chains and are therefore presented in both *A* and *B*. (†) MRL2-57 uses a γ constant region associated with the VH3H9 tg. Nucleotide sequence for these L chains are available from EMBL/GenBank/DDBJ under accession numbers U18561–U18599; U19308–U19343.

ation with the VH3H9 tg. In addition to the Vκ12a, several other V κ genes (e.g., 2a, 4f, 9c, 34a) are also present in both hybridoma panels (Figs. 3 *A* and 4). That several L chain genes are shared argues for a similar available repertoire of L chain genes between BALB/c and MRL-*lpr/lpr* mice.

Anti-HN Igs from lpr/lpr Mice Use Distinct L Chain Gene Segments. In general, the anti-HN Igs from the MRL-*lpr/lpr* VH3H9 tg hybridomas use L chain V gene segments not used in the BALB/c VH3H9 tg hybridomas (Figs. 3 and 4). Many of the L chains used in the HN set have been previously identified, paired with a variety of H chains, in anti-DNA Igs from several murine SLE models (Fig. 5). This suggests that these L chains, like the VH3H9 H chain, have inherent DNA-binding ability. The fact that we can rescue VH3H9 tg plus endogenous L chain pairs that generate anti-HN Igs from MRL-*lpr/lpr* VH3H9 tg mice rules out the trivial explanation that their absence in the BALB/c VH3H9 tg panels is the consequence of the inability of these H+L chain pairs to form. Instead, the absence of particular L chains from the BALB/c VH3H9 tg hybridomas suggests that B cells bearing these L chains with the VH3H9 tg are negatively selected as a consequence of their self-reactivity.

Analysis of the usage of the Vλ1 L chain gene segment in the hybridoma panels provides clear evidence that VL genes that can pair with VH3H9 to give anti-HN Igs are selected against in BALB/c mice but not in MRL-*lpr/lpr* mice. Transfection experiments using the VH3H9 H chain into a BALB/c-derived cell line that expresses a germline Vλ1 have shown that Vλ1/VH3H9 Igs are anti-HN (49). Eight of nine λ1-expressing hybridomas from the MRL-*lpr/lpr* VH3H9 tg mice express a Vλ1/VH3H9 combination, and the hybridomas are anti-HN. In contrast, there is only one example of a Vλ1/VH3H9 tg hybridoma retrieved from BALB/c VH3H9 tg mice. This hybridoma does not display HN reactivity, apparently due to coexpression of a κ protein (Fig. 3 *B*). One of the Vλ1 MRL-*lpr/lpr* VH3H9 tg-derived hybridomas also coexpresses a κ protein and is likewise not anti-HN (Fig. 3 *B*). Although the mechanism by which coexpression of an additional L chain interferes with the anti-HN specificity of hybridomas that also contain the Vλ1/VH3H9 combination has not been established, this example underscores that BALB/c VH3H9 tg mice can rearrange at least one anti-HN–associated gene (Vλ1). However, its expression appears to be limited to B cells that coexpress an additional L chain that disrupts its specificity for DNA.

J κ Gene Use in VH3H9 tg Hybridomas. A second example that highlights that BALB/c VH3H9 tg mice have the genetic capacity to produce anti-HN Igs is the use of the Vκ12d gene segment. A set of hybridomas from both BALB/c and MRL-*lpr/lpr* VH3H9 tg mice use the same Vκ12d V gene segment but differ in their specificity for DNA (Fig. 3 *A*). Three of the four Vκ12d/VH3H9 tg hybridomas derived from the MRL-*lpr/lpr* background are anti-HN. In contrast, the single Vκ12d/VH3H9 tg hybridoma derived from the BALB/c background only binds ssDNA, as does the fourth MRL-*lpr/lpr* hybridoma. Although the Vκ12d V gene segments for all of these hybridomas are 100% identical at the nucleotide level over the region sequenced (compare Vκ12d sequences from Fig. 3 *A*), there are differences in the J κ gene segments used. The three anti-HN MRL-*lpr/lpr* VH3H9 tg hybridomas use Jκ2′. The ssDNA binding hybridoma from the BALB1 VH3H9 tg mouse uses Vκ12d recombined to a Jκ1, as does the ssDNA binding MRL3 hybridoma. As shown in Fig. 3 *A*, the BALB1 VH3H9 tg mouse can and does use Jκ2′ with other VLs, but does not with Vκ12d, further underscoring the negative selection of B cells bearing the anti-HN specificity in nonautoimmune mice.

Several studies have proposed that developing B cells that are autoreactive can be rescued from deletion by revising their Ig receptors. The uncoupling of the fate of the B cell from its antigen receptor has been termed receptor editing (25, 45, 50). We have evaluated the BALB1 VH3H9 tg hybridomas described in this study for evidence of receptor editing. Replacement of a primary κ rearrangement with a secondary rearrangement on the same allele would predict the accumulation of 3′ J κs in the nonautoreactive B cells (25, 50). Indeed, a bias in the 3′ Jκ5 gene segment usage was observed in an earlier examination of BALB/c VH3H9 tg-derived hybridomas and was, therefore, interpreted as evidence for receptor editing (25). However, no 3′ J κ bias was observed in this current examination of κ gene segment usage in BALB1 VH3H9 tg-derived hybridomas (Fig. 3). It is possible that the different protocols used to generate the hybridoma panels may be responsible for this difference in J κ gene segment

		FW1	CDR1	FW2	CDR2	FW3	CDR3	FW4	Jk	V heavy	ss/dsDNA
V Kappa 4/5											
	DNA22	QIVLTQSPAIMSASPGERVTMTC	SASSSVSSSYLY	WYQQKPGSSPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQYSGYPFT	FGTG	4′	μJ558	
G	MRL1-56		---	---------------	-------	--------------------------------	-------S-	--S-	2′	3H9tg	+/HN
V Kappa 9											
	DP7VK	DIQMTQSPSSLSASLGERVSLTC	RASQDIDSSLN	WLQQEPDGTIKRLIY	ATSSLDS	GVPKRFSGSRSGSDYSLTISSLESEDFVDYYC	LQYASSPWT	FGGG	1	γJ558	
C	MRL2-169	-	------G----	---------------	--R----	---E----------------------------	Q--G-F-L-	--A-	5	3H9tg	+/HN
V Kappa 12/13											
	185-c1	DIQMTQSPASLSASVGETVTITC	RASENIYSYLA	WYQQKQGKSPQLLVY	NAKTLAE	GVPSRFSGSGSGTQFSLKINSLQPEDFGSYYC	QHHYGTPPT	FGGG	1	γQ52	
D	MRL3-34	------------	-----------	---------------	-------	--------------------------------	-------Y-	--S-	2′	3H9tg	+/HN
V Kappa 23											
	DP12	DIVLTQSPATLSVTPGDRVSLSC	RASQSISNYLH	WYQQKSHESPRLLIK	YASQSIS	GIPSRFSGSGSGTDFTLSINSVETEDFGMYFC	QQSNSWPYT	FGSG	2′	μ7183	
A	MRL2-171	------------	-------K---	---------------	-------	--------------------------------	---K---L-	--A-	5	3H9tg	+/HN
Lambda 1											
	165.33	QAVVTQDSALTTSPGETVTLTC	RSSTGAVTTSNYAN	WVQEKPDHLFTGLIG	GTNNRAP	GVPARFSGSLIGDKAALTITGAQTEDEAIYFC	ALWYSNHWV	FGGG	1	μQ52	
	MRL3-21	----------------------	--------------	---------------	-------	--------------------------------	---------	----	1	3H9tg	+/HN

Figure 5. Anti-HN Igs from VH3H9 tg MRL-*lpr/lpr* mice use a set of L chains previously described in nontransgenic anti-DNA antibodies from MRL-*lpr/lpr* and (NZB × NZW) mice. The anti-HN V L/VH3H9 tg sequences are compared with similar anti-DNA antibody sequences from autoimmune-prone nontransgenic mice. Reference sequences were as follows: DNA22 (40); 3H9 (24, 49); DP7, DP12 (24); and 185-c1, 165.33 (41).

usage: the earlier hybridomas were generated from LPS-activated B cells (25), whereas the hybridomas reported here were derived from unmanipulated spleen cells. The data presented here do not suggest a role for receptor editing in the elimination of anti-HN cells from BALB/c mice.

Is the Fas-defect in MRL-lpr/lpr Mice Responsible for the Breakdown in B Cell Tolerance? The *lpr* mutation in the *fas* gene is an obvious candidate for mediating the breakdown in B cell tolerance observed in MRL-*lpr/lpr* mice. A number of different strains homozygous for the *lpr* mutation develop anti-DNA antibodies (51). In addition we detected anti-HN B cells in our earliest backcross mice (backcross 2 onto MRL-*lpr/lpr*, Tables 1 and 2). The role of Fas in murine lupus is unclear; it could be exerting its effects via the "auto-antigen" and/or directly in the autoreactive B cell. Recently, it was reported that the majority of auto-antigens targeted in SLE are found reorganized and clustered within distinct cell surface structures during cell death by apoptosis (52). Given Fas' link to apoptosis (53), a defective Fas may manifest itself as a defect in apoptosis. It has been proposed that failure of Fas-mediated T cell apoptosis within the thymus of *lpr/lpr* mice may facilitate the persistence of autoreactive cells and may at the same time also deprive the animal of the "apoptotic" auto-antigens that serve as tolerogens (52).

We would extend this model to include an effect of the *lpr* mutation on B cell negative selection. If apoptotic bodies are the tolerogen responsible for mediating B cell deletion in normal mice, MRL-*lpr/lpr* mice may exhibit altered tolerogen expression due to the Fas defect which may in turn interfere with anti-HN B cell deletion. At the same time, however, elegant experiments using allophenic mice strongly argue for an intrinsic defect in *lpr/lpr* B cells. In these studies, where the B cells were either of the *lpr/lpr* or non-*lpr/lpr* genotype and the antigenic environment was shared, only the *lpr/lpr* B cells contributed to serum autoantibodies (2, 3, 6). It has also been reported that MRL/MpJ-+/+ mice, which are identical to MRL-*lpr/lpr* mice except for the *lpr* mutation, develop anti-DNA Igs and other symptoms of lupus, but with a delayed onset relative to MRL-*lpr/lpr* mice (54). To help resolve this issue, we are extending our analysis to include MRL/MpJ-+/+ VH3H9 tg mice.

In conclusion, we hypothesize that the *lpr* mutation may disrupt both the B cell's susceptibility to negative selection and the quality and quantity of the tolerogen. Both of these alterations may be required for the production of the specific set of autoantibodies found in MRL-*lpr/lpr* mice.

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References

1. Tan, E.M. 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* 44:93–151.
2. Perkins, D.L., R.M. Glaser, C.A. Mahon, J. Michaelson, and A. Marshak-Rothstein. 1990. Evidence for an intrinsic B cell defect in *lpr/lpr* mice apparent in neonatal chimeras. *J. Immunol.* 145:549–555.
3. Nemazee, D., C. Guiet, K. Buerki, and A. Marshak-Rothstein. 1991. B lymphocytes from the autoimmune-prone mouse strain MRL/*lpr* manifest an intrinsic defect in tetraparental MRL/*lpr* < - > DBA/2 chimeras. *J. Immunol.* 147:2536–2539.
4. Katagiri, T., S. Azuma, Y. Toyoda, S. Mori, K. Kano, P.L. Cohen, and R.A. Eisenberg. 1992. Tetraparental mice reveal complex cellular interactions of the mutant, autoimmunity-inducing *lpr* gene. *J. Immunol.* 148:430–438.
5. Sobel, E.J., T. Katagiri, K. Kagiri, S.C. Morris, P.L. Cohen, and R.A. Eisenberg. 1991. An intrinsic B cell defect is required for the production of autoantibodies in the *lpr* model of murine systemic autoimmunity. *J. Exp. Med.* 173:1441–1449.
6. Sobel, E.S., P.L. Cohen, and R.A. Eisenberg. 1993. *lpr* T cells are necessary for autoantibody production in *lpr* mice. *J. Immunol.* 150:4160–4167.
7. Nemazee, D.A., and K. Bürki. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature (Lond.).* 337:562–566.
8. Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.A. Brink, H. Pritchard-Briscoe, J.S. Wothersponn, R.H. Loblay, K. Raphael, et al. 1988. Altered immunoglobulin expression and function silencing of self-reactive B lymphocytes in transgenic mice. *Nature (Lond.).* 334:676–682.
9. Hardin, J.A., and J.E. Craft. 1987. Patterns of autoimmunity to nucleoproteins in patients with systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* 13:37–46.
10. Andrews, B.S., R.A. Eisenberg, A.N. Theofilopoulos, S. Izui, C.B. Wilson, P.J. McConahey, E.D. Murphy, J.B. Roths, and F.J. Dixon. 1978. Spontaneous murine lupus-like syndromes.

J. Exp. Med. 148:1198–1215.
11. Theofilopoulos, A.N., R. Kofler, P.A. Singer, and F.J. Dixon. 1989. Molecular genetics of murine lupus models. *Adv. Immunol.* 46:61–109.
12. Cohen, P.L., and R.A. Eisenberg. 1991. *Lpr* and *gld*: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* 9:243–269.
13. Wallach, J. 1992. Interpretation of Diagnostic Tests: A Synopsis of Laboratory Medicine. Little, Brown and Company, Boston. 694–698.
14. Watanabe-Fukunaga, R., C.I. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature (Lond.).* 356:314–317.
15. Wu, J., T. Zhou, J. He, and J.D. Mountz. 1993. Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. *J. Exp. Med.* 178:461–468.
16. Chu, J.-L., J. Drappa, A. Parnassa, and K.B. Elkon. 1993. The defect of Fas mRNA expression in MRL/*lpr* mice is associated with insertion of the retrotransposon, *ETn*. *J. Exp. Med.* 178:723–730.
17. Drappa, J., N. Brot, and K.B. Elkon. 1993. The Fas protein is expressed at high levels of $CD4^+CD8^+$ thymocytes and activated mature lymphocytes in normal mice but not in the lupus-prone strain MRL *lpr/lpr*. *Proc. Natl. Acad. Sci. USA.* 90:10340–10344.
18. Tsao, B.P., A. Chow, H. Cheroutre, Y.W. Song, M.E. McGrath, and M. Kroneberg. 1993. B cells are anergic in transgenic mice that express IgM anti-DNA antibodies. *Eur. J. Immunol.* 23: 2332–2339.
19. Singer, P.A., and A.N. Theofilopoulos. 1990. T-cell receptor Vβ repertoire expression in murine models of SLE. *Immunol. Rev.* 118:103–127.
20. Herron, L.R., R.A. Eisenberg, E. Roper, V.N. Kakkanaiah, P.L. Cohen, and B. Kotzin. 1993. Selection of the T cell receptor repertoire in *lpr* mice. *J. Immunol.* 151:3450–3459.
21. Zhou, T., H. Bluethmann, J. Zhang, C.K. Edwards III, and J.D. Mountz. 1992. Defective maintenance of T cell tolerance to a superantigen in MRL-*lpr/lpr* mice. *J. Exp. Med.* 176: 1063–1072.
22. Scott, D.E., W.J. Kisch, and A.D. Steinberg. 1993. Studies of T cell deletion and T cell anergy following in vivo administration of SEB to normal and lupus-prone mice. *J. Immunol.* 150:664–672.
23. Izui, S., V.E. Kelley, K. Masuda, H. Yoshida, J.B. Roths, and E.D. Murphy. 1984. Induction of various autoantibodies by mutant gene *lpr* in several strains of mice. *J. Immunol.* 133: 227–233.
24. Shlomchik, M., M. Mascelli, H. Shan, M.Z. Radic, D. Pisetsky, A. Marshak-Rothstein, and M. Weigert. 1990. Anti-DNA antiodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med.* 171:265–297.
25. Radic, M.Z., J. Erikson, S. Litwin, and M. Weigert. 1993. B lymphocytes may escape tolerance by revising their antigen receptors. *J. Exp. Med.* 177:1165–1173.
26. Erikson, J., M.Z. Radic, S.A. Camper, R.R. Hardy, C. Carmack, and M. Weigert. 1991. Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. *Nature (Lond.).* 349:331–334.
27. Kohler, G. 1980. Immunoglobulin chain loss in hybridoma lines. *Proc. Natl. Acad. Sci. USA.* 77:2197–2199.
28. Marcu, K.B., J. Banerji, N.A. Penncavage, R. Lang, and N. Arheim. 1980. 5′ flanking region of immunoglobulin heavy chain constant region genes displays length heterogeneity in germlines of inbred mouse strains. *Cell.* 22:187–196.
29. Van Ness, B.G., C. Coleclough, R.P. Perry, and M. Weigert. 1982. DNA between variable and joining gene segments of immunoglobulin κ light chain is frequently retained in cells that rearrange the κ locus. *Proc. Natl. Acad. Sci. USA.* 79: 262–266.
30. Caton, A.J., S.E. Stark, J. Kavaler, L.M. Staudt, D. Schwartz, and W. Gerhard. 1991. Many variable region genes are utilized in the antibody response of BALB/c mice the influenza virus A/PR/8/34 hemagglutinin. *J. Immunol.* 147:1675–1686.
31. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.
32. Kabat, E.A., T.T. Wu, H.M. Perry, K.S. Gottesman, and C. Foeller. 1991. Sequences of proteins of immunological interest. *U.S. Department of Health and Human Services.* 1–2592.
33. Huse, W.D., L. Sastry, S.A. Iverson, A.S. Kang, M. Alting-Mees, D.R. Burton, S.J. Benkovic, and R.A. Lerner. 1989. Generation of a large combinatorial library of the immunoglobulin repertoire in phage lambda. *Science (Wash. DC).* 246: 1275–1281.
34. Bernard, O., N. Hozumi, and S. Tonegawa. 1978. Sequences of mouse immunoglobulin light chain genes before and after somatic changes. *Cell.* 15:1133–1144.
35. Tonegawa, S., A.M. Maxam, R. Tizard, O. Bernard, and W. Gilbert. 1978. Sequences of a mouse germ-line gene for a variable region of an immunoglobulin light chain. *Proc. Natl. Acad. Sci. USA.* 75:1485–1489.
36. Nadel, B., A.-M. Drapier, L. Andrade, P.-A. Cazenave, and P. Sanchez. 1991. Preferential VH/Vλ pairings occur in the available B cell repertoire of adult BALB/c mice. *Int. Immunol.* 4:501–507.
37. Caton, A.J., G.G. Brownlee, L.M. Staudt, and W. Gerhard. 1986. Structural and functional implications of a restricted antibody response to a defined antigenic region on the influenza virus hemagglutinin. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:1577–1587.
38. Kavaler, J., A.J. Caton, L.M. Staudt, D. Schwartz, and W. Gerhard. 1990. A set of closely related antibodies dominates the primary antibody response to the antigenic site CB of the A/PR/8/34 influenza virus hemagglutinin. *J. Immunol.* 145: 2312–2321.
39. Ohnishi, K., F.M. Ebling, B. Mitchell, R.R. Singh, B.H. Hahn, and B.P. Tsao. 1994. Comparison of pathogenic and nonpathogenic murine antibodies to DNA: antigen binding and structural characteristics. *Int. Immunol.* 6:817–830.
40. Kofler, R., R. Strohal, R.A. Balderas, M.E. Johnson, D.J. Noonan, M. Duchosal, F.J. Dixon, and A.N. Theofilopoulos. 1988. Immunoglobulin κ light chain variable region gene complex organization and genes encoding anti-DNA autoantibodies in lupus mice. *J. Clin. Invest.* 82:852–860.
41. Tillman, D.M., N.-T. Jou, R.J. Hill, and T.N. Marion. 1992. Both IgM and IgG anti-DNA antibodies are the products of clonally selective B cell stimulation in (NZB × NZW)F_1 mice. *J. Exp. Med.* 176:761–779.
42. Conger, J.D., B.L. Pike, and G.J.V. Nossal. 1987. Clonal analysis of the anti-DNA repertoire of murine B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 84:2931–2935.
43. Pisetsky, D.S., and S.A. Caster. 1982. The B-cell repertoire for autoantibodies: frequencies of precursor cells for anti-DNA

antibodies. *Cell. Immunol.* 72:294–305.
44. Offen, D., L. Spatz, H. Escowitz, S. Factor, and B. Diamond. 1992. Induction of tolerance to an IgG autoantibody. *Proc. Natl. Acad. Sci. USA.* 89:8332–8336.
45. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J. Exp. Med.* 177:999–1008.
46. Chen, C., M.Z. Radic, J. Erikson, S.A. Camper, S. Litwin, R.R. Hardy, and M. Weigert. 1994. Deletion and editing of B cells that express antibodies to DNA. *J. Immunol.* 152: 1970–1982.
47. Kalled, S.L., and P.H. Brodeur. 1990. Preferential rearrangement of Vκ4 gene segements in pre-B cell lines. *J. Exp. Med.* 172:559–566.
48. Milstein, C., J. Even, J.M. Jarvis, A. Gonzalez-Fernandez, and E. Gherardi. 1992. Non-random features of the repertoire expressed by members of one Vκ gene family and of the V-J recombination. *Eur. J. Immunol.* 22:1627–1634.
49. Radic, M.Z., M.A. Mascelli, J. Erikson, H. Shan, and M. Weigert. 1991. Ig H and L chain contributions to autoimmune specificities. *J. Immunol.* 146:176–182.
50. Tiegs, S.L., D.M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med.* 177: 1009–1020.
51. Itoh, N., Y. Tsujimoto, and S. Nagata. 1993. Effect of bcl-2 on Fas antigen-mediated cell death. *J. Immunol.* 151:621–627.
52. Casciola-Rosen, L.A., G. Anhalt, and A. Rosen. 1994. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* 179:1317–1330.
53. Trauth, B.C., C. Klas, A.M.J. Peters, S. Matzku, P. Moller, W. Falk, K.-M. Debatin, and P.H. Krammer. 1989. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science (Wash. DC).* 245:301–305.
54. Murphy, E.D., and J.B. Roths. 1978. A new congenic inbred strain: MRL/Mp-*lpr/lpr*, *lpr/+*, +/+. *Mouse News Letter.* 58:51.
55. Max, E.E., J.V. Maizel, and P. Leder. 1981. The nucleotide sequence of a 5.5-kilobase cDNA segment containing the mouse κ immunoglobulin J and C region genes. *J. Biol. Chem.* 256: 5116–5120.
56. Boyd, R.T., M.M. Goldrick, and P. Gottlieb. 1986. Genetic polymorphism at the mouse immunoglobulin Jκ locus (Igκ-J) as demonstrated by Southern hybridization and nucleotide sequence analysis. *Immunogenetics.* 24:150–157.