Both IgM and IgG Anti-DNA Antibodies Are the Products of Clonally Selective B Cell Stimulation in (NZB × NZW)F1 Mice

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Summary

Disease activity in systemic lupus erythematosus is closely associated with the appearance of immunoglobulin (Ig)G antibody to native DNA in both humans and mice. Like normal antibody responses, the anti-DNA autoantibody first appears as IgM and then switches to IgG. Structural studies of IgG anti-DNA suggest that these antibodies are the products of clonally selected, specifically stimulated B cells. The origins of the IgM anti-DNA have been less clear. To determine whether the earlier appearing IgM anti-DNA antibody in autoimmune mice also derives from clonally selected, specifically stimulated B cells or B cells activated by nonselective, polyclonal stimuli, we have analyzed the molecular and serological characteristics of a large number of monoclonal IgM anti-DNA antibodies from autoimmune (NZB × NZW)F1 mice. We have also analyzed IgM and IgG anti-DNA hybridomas obtained from the same individual mice to determine how the later-appearing IgG autoantibody may be related to the earlier-appearing IgM autoantibody within an individual mouse. The results demonstrate that: (*a*) IgM anti-DNA, like IgG, has the characteristics of a specifically stimulated antibody; (*b*) IgM and IgG anti-DNA antibodies have similar variable region structures and within individual mice may be produced by B cells derived from the same clonal precursors; (*c*) recurrent germline and somatically derived VH and VL structures may influence the specificity of anti-DNA monoclonal antibody for denatured vs. native DNA; and (*d*) the results provide a structural explanation for the selective development of IgG antibody to native DNA as autoimmunity to DNA progresses in (NZB × NZW)F1 mice.

Antibody to DNA is a manifestation of the autoimmune disease SLE and plays a demonstrable role in disease pathogenesis in both humans (1) and (NZB × NZW)F1 mice (2, 3). Because of similarities to humans in the development of disease, (NZB × NZW)F1 mice have become useful experimental models for studying the cellular and molecular basis for anti-DNA autoantibody production (3). In particular, the availability of hybridoma-derived, anti-DNA mAbs from autoimmune mice has provided the opportunity to analyze the structural basis for antibody specificity to DNA (4). How anti-DNA antibody originates both in human and mouse SLE remains obscure, however.

Previous experiments from our laboratories have concentrated on the analysis of interclonal and intraclonal diversity of spontaneous anti-DNA antibodies within an individual autoimmune (NZB × NZW)F1 mouse (5–7). The results from those analyses demonstrated that the IgG anti-DNA antibody within an individual autoimmune mouse had all the characteristics attributable to clonally selected, secondary immune antibody to specific antigen: IgG isotype, oligoclonality, and V region somatic mutations among clonally related antibodies. The results also suggested that the antigen most likely to have stimulated such an antibody was DNA. In this regard, anti-DNA in (NZB × NZW)F1 mice appears to be similar to anti-DNA in MRL *lpr/lpr* mice (8, 9). However, the results from at least one study on the clonal heterogeneity of anti-DNA antibody within individual autoimmune (NZB × NZW)F1 mice led to a different conclusion (10). Although V_H[1] and V_L gene representation among the hybridomas in the previous study was not consistent with a polyclonal population, the hybridomas were nevertheless clonally diverse. Anti-DNA antibody in autoimmune mice undergoes a spontaneous isotype shift from IgM to IgG (11–13). Analyses of B cell activity in (NZB × NZW)F1

[1] *Abbreviations used in this paper:* VH, immunoglobulin heavy chain variable region; V_H, heavy chain variable region gene; VL, immunoglobulin light chain variable region; V_L, light chain variable region gene.

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mice (14) have led to the hypothesis that at least the initial, IgM stage of anti-DNA autoantibody production is due to polyclonal activation of B cells (15) and that subsequent clonal selection of IgG anti-DNA-producing B cells occurs subsequent to this event. However, there have been no direct studies to determine whether IgM anti-DNA autoantibodies have the characteristics of antibody produced by a nonselected, polyclonal population of B cells. Likewise, there have been no studies to directly determine the clonal relationship between the IgM and IgG anti-DNA autoantibodies within individual (NZB × NZW)F_1 mice.

The present experiments were proposed to accomplish three major goals. (*a*) Since there has been some question as to whether the autoimmune anti-DNA antibody in (NZB × NZW)F_1 mice is generally oligoclonal and characteristic of an antigen-stimulated, secondary immune response, we extended our analysis of the clonal diversity of IgG anti-DNA antibodies to include seven additional (NZB × NZW)F_1 mice. (*b*) To determine whether the earlier-appearing IgM anti-DNA antibody is also clonally selected and expresses V region structures that are similar to the later-appearing IgG anti-DNA antibodies in autoimmune (NZB × NZW)F_1 mice, a large number of IgM anti-DNA hybridomas from four different mice were analyzed. (*c*) To determine whether the IgG anti-DNA antibody within an individual autoimmune mouse is clonally related to the earlier-appearing IgM anti-DNA antibody within the same mouse, IgM and IgG hybridomas from three individual mice were analyzed. The results confirmed our previous results that spontaneous IgG anti-DNA antibodies in (NZB × NZW)F_1 mice are generally oligoclonal in origin and have V region structural characteristics consistent with an antigen-selected derivation. The results also demonstrated preferential expression of particular V_H and V_L genes among IgM anti-DNA hybridomas, suggesting that IgM anti-DNA is also produced by selective B cell stimulation. Finally, the results demonstrated that in general IgM and IgG anti-DNA autoantibody-producing B cells have structurally similar Ig V regions and that within an individual mouse at least some of the IgG anti-DNA antibody-producing B cells are derived from the same clonal precursors as the IgM anti-DNA antibody-producing B cells.

Materials and Methods

Mice and Generation of Hybridomas. (NZB × NZW)F_1 mice were purchased from Harlan/Sprague-Dawley (Indianapolis, IN) and maintained in a pathogen-free environment within the animal facilities at The University of Tennessee, Memphis. Hybridomas were generated as described previously (5). A partial splenectomy and B cell fusion were performed on one mouse. The mouse was completely anesthetized by a combination of intraperitoneal administration of a mixture of xylazine/ketamine/butorphanol (25:40:4 mg/kg body weight) and intermittent inhalation of metaphane throughout the surgical procedure. An incision was made in the left flank. The blood vessels in the vascular bundle that enters the hilus of the spleen were simultaneously sealed and cauterized. The spleen was cut and the free half removed and used to generate hybridomas. The abdominal wall and overlying skin were closed with surgical staples that were removed 10 d after surgery. The remaining half of the spleen was removed at a later date for hybridoma production by the normal procedure.

ELISA for Anti-DNA. The direct-binding, solid-phase ELISA used to detect anti-DNA-positive culture wells after the fusion and determination of IgH and L isotypes have been described in detail (5). Culture wells were chosen for cloning of hybridomas only when the relevant culture supernatant produced a relative antibody activity of 3 on a scale of 1 to 10, with 10 being the maximum activity of the assay ($OD_{405} \geq 1.2$). The supernatants were also screened for binding to micro-ELISA plates that were not coated with DNA but were treated identically otherwise. The source of DNA used for screening hybridoma supernatants was commercial calf thymus DNA sheared by sonication. Only hybrids producing antibody that was positive on DNA-coated plates and negative for binding to plates not coated with DNA were considered for cloning. This screening procedure allowed us to select for hybridomas producing both low- and high-avidity IgM and IgG DNA binding antibodies, as indicated by the data in Table 1. The assay did not discriminate between ssDNA and dsDNA specificity.

cDNA Sequencing. The procedures for isolation of mRNA (16) and cDNA sequencing (17), the sequences of the oligonucleotides used as primers, and the method for sequence comparisons were exactly the same as those we have used previously (7).

Statistical Analyses. The numbers of germline V_H and V_L genes that could encode anti-DNA were estimated from the repeat frequencies of individual V_H and V_L observed among all the different clones represented in this study. V_H and V_L from individual clones were considered to have been derived from the same germline V_H or V_L if the nucleotide sequences were ≥95% homologous. The identical pair method of Briles and Carroll (18) was used to obtain the estimates: $R = N/A$, where $N = n(n-1)/2$; $A = \sum_{i=1}^{m} \alpha_i(\alpha_i - 1)/2$; n = number of dependent sequences; and α_i = number of sequences derived from the same germline sequence i. The number of dependent sequences (n) used to calculate the estimates was adjusted to account for repeat frequencies among either V_H or V_L sequences that deviated from the expected normal distribution (19). For V_L, $n = 42$, $A = 14$, and $N = 861$; therefore, the estimated number of V_L in the anti-DNA repertoire (R) is 62 (95% confidence interval [CI] = 43, 76). For V_H, $n = 55$, $A = 34$, and $N = 1{,}485$; therefore, $R = 44$ (95% CI = 31 and 63). For V_H558, $n = 28$, $A = 16$, and $N = 378$; therefore, $R = 24$ (95% CI = 15 and 36). Calculations of the probabilities that individual V_H or V_L genes, or V_H-V_L combinations, would be expressed at the indicated frequencies in the absence of selection among the population of hybridomas assumed a binomial distribution for any given V gene (20).

Results

Oligoclonality of IgG Anti-DNA Hybridomas. The criteria used in choosing mice for spleen cell fusions to generate hybridomas were age, serum titer, and isotype of spontaneous anti-DNA antibody. Mice used to generate IgM hybridomas were <6 mo of age, with IgG anti-DNA titers ≤90 and IgM anti-DNA titers ≥1,000. Mice chosen for generating IgG anti-DNA hybridomas were usually 6–8 mo old or older, with serum IgG anti-DNA titers ≥1,000. Hybridomas generated from the fusions were chosen for cloning as described in Materials and Methods. This procedure was specifically chosen so as not to bias the population of hybridomas, particularly the IgM, toward either low- or high-affinity antibody. Specificity analyses of the mAbs in competitive ELISAs confirmed that there was no bias in the selection procedure for hybridomas producing either high- or low-affinity antibody (Table 1).

During the course of this study, 107 anti-DNA antibody-producing hybridomas from 10 different autoimmune (NZB × NZW)F_1 mice were generated. Each hybridoma was analyzed for VH and VL cDNA nucleotide sequences (Table 1, and Figs. 1 and 2) and for isotype and DNA specificity of its respective mAb. In each mouse with predominantly IgG serum anti-DNA (mouse nos. 17, 111, 163, 10, 74, 83, and 185), the majority of the IgG hybridomas formed clonally related groups. For the purpose of comparing the clonal repertoire between IgM and IgG anti-DNA antibodies among different individual mice, data in Table 1 and Figs. 1 and 2 are from only one representative hybridoma of a given clonally related group. Clones with more than one hybridoma are indicated with a "c" designation as part of the clone number (e.g., 111-c1). For clones with more than one hybridoma, the number of hybridomas in each clone are indicated in Table 1 and Figs. 1 and 2. Clonal relatedness among relevant hybridomas was confirmed by nucleotide sequences, particularly in the junctional regions between V and D and D and J of the heavy chain, and V and J of the light chain, and by the identity of productive and nonproductive J_H and J_κ rearrangements within each clonal member (21). There were 38 IgM hybridomas from 36 clones, and 69 IgG hybridomas from 29 clones (Table 1). Two clones had IgM and IgG hybridomas from the same clone. The degree to which individual clones were expanded in each of the mice was quite variable, from single representatives such as 17s.83 and 163.42 to clones with nine representative hybridomas such as clones 163-c1 and 185-c1 (Table 1). There was considerable intraclonal variation due to somatic mutation in each of the multiple member clones ("c" clones in Table 1). Detailed analyses of the mutations and their effects on the specificity of the respective antibodies will be presented and discussed elsewhere (N.-T. Jou, D. Tillman, R. Hill, and T. Marion, manuscript in preparation).

Recurrent V_H Gene Usage among Both IgM and IgG Anti-DNA. Multiple clones from two or more animals expressed at least one V_H gene from each of the V_H558, V_H7183, V_HQ52, and V_HS107 germline V_H families (Table 1). For example, 165.14 and 17s.128 each expressed a V_H558 family V_H gene that is nearly identical to the previously identified V_H for the anti-DNA hybridoma BXW-DNA16 (Fig. 1 *A*, *BWDNA16*) (22). Likewise, 17s-c1 and 165.60 had nearly identical V_H that are also similar to V_H558-*BWDNA16*, as did 10-c1 and 17s.166, and 163.72 and 25.12m. Extreme examples of identical or nearly identical V_H gene expression are clones 111.185, 165.27, 165.49, and 17s.83. These clones expressed a V_H558 family V_H gene very similar to that expressed by the previously identified anti-DNA hybridoma MLR-DNA22 (Fig. 1 *A*, *DNA22*) (22). Eight clones expressed a V_H previously identified for the hybridoma BXW-DNA7 (Fig. 1 *A*, *BWDNA7*). In the latter two groups of anti-DNA clones, four clones from three different mice (Fig. 1 *A*, *DNA22*) and eight clones from four different mice (Fig. 1 *A*, *BWDNA7*), respectively, use the same V_H gene.

The repetitive usage of V_H genes was apparent among both IgM- and IgG-producing clones for each of the V_H genes described above. For example, 111.185 and 165.27 are IgM, and 165.49 and 17s.83 are IgG (Table 1 and Fig. 1 *A*, *DNA22*). Likewise, 17p.101, 202.80, 202.s38, 202.135, 202.61, 165.3m, and one of the hybridomas in 111-c1 are IgM (Table 1 and Fig. 1 *A*, *BWDNA7*). All the hybridomas in 111-c2 and three hybridomas in 111-c1 were IgG. Clone 17ps-c7 had two IgM hybridomas and one IgG hybridoma with a V_H gene from the V_H10 family (Table 1 and Fig. 3). In almost every case, at least one IgM and one IgG hybridoma expressed the same V_H gene, usually with a different D_H. Notable exceptions were two IgM-producing hybridomas, one of which had a VH derived from the V_H606 family and the other from the V_H36-60 family (Table 1). These two V_H gene families were not represented among any of the IgG-producing hybridomas. Likewise, hybridomas expressing a V_H from the V_HS107 family were found only among IgG-producing hybridomas (Table 1 and Fig. 2).

The estimated number of different germline V_H genes that could encode an anti-DNA antibody is 44 (see Materials and Methods). The probability that four different clones out of the total of 63 would express a VH derived from the same germline V_H gene by chance alone is 0.042. These results indicate that among the total population of clones there was preferential expression of V_H genes homologous to the V_H558 family genes expressed by the anti-DNA hybridomas MRL-DNA22 (111.185, 165.27, 165.49, 17s.83; Fig. 1 *A*, *DNA22*) and BWDNA7 (111-c1, 111-c2, 17p.101, 202.80, 202.s38, 202.135, 202.61, and 165.3m, 13 hybridomas total; Fig. 1 *A*, *BWDNA7*) (22). There was also preferential usage of a V_HQ52-derived V_H gene for which homologous V_H genes have not been reported (Fig. 1 *C*).

Both the IgM and IgG anti-DNA hybridoma populations independently demonstrated preferential V_H gene expression. The probability that three IgM clones cut out of the total of 36 would express a VH derived from the same germline V_H gene by chance is 0.020. Therefore, there was a strong preference among the IgM hybridomas for V_H genes homologous to genes from the V_HQ52 family (four clones: 165.33, 165.41, 165.52, and 202.17; Fig. 1 *C*). There was also preferential usage of a gene from the V_H558 family similar to the V_H gene used by the anti-DNA hybridoma BXW-DNA7 (22) (six clones: 17p.101, 202.80, 202.s38, 202.135, 202.61, and 165.3m; Fig. 1 *A*, *BWDNA7*). IgM clones 165.60, 163.72, and 25.12m are ⩾92% homologous and use a V_H558 family gene homologous to the previously described V_H for the anti-DNA hybridoma BXW-DNA16 (22).

As stated in the previous section, most of the hybridomas producing IgG anti-DNA was found to be members of clones represented by two or more hybridomas. Of the 29 clones represented among the 69 IgG hybridomas, 18 were represented by two or more hybridomas. If the analysis of preferential V_H gene usage is determined on the basis of the frequency that a particular V_H gene is used among IgG clones, there was preferential expression ($p < 0.025$) of a V_H homologous to the V_H558 Vh31 germline gene (17s-c2, 165.3, and 74-c1; Fig. 1 *A*, *S57[Vh31]*); and the V_HS107 family Vh11

Table 1. *Summary of Variable Region Structures and DNA Specificity for Monoclonal Anti-DNA Autoantibodies*

			VH			VL		DNA binding specificity*		
Mouse	Clone[‡]	Isotype	V_H[§]	D_H[‖]	J_H	V_L[¶]	J_L	ssDNA	dsDNA	CDLP**
17	p101	IgM	V_H558	LI	2	Vκ-1	1	8.84	NI*	NB
	p73	IgM	V_H7183	RDN	4	Vκ-21	1	>10	NI	<2
	p3	IgM	V_H606	TP	2	Vκ-2	1	EB*	NI	NB
	s93m	IgM	V_H7183	RGTTVY	2	Vκ-1	5	1.39	*15%*	NB
	s128	IgM	V_H558	ALRQGY	2	Vκ-21	2	7.43	*19*	NB
	s13	IgM	V_H558	SRGYYFGSSRF	1	Vκ-1	1	0.46	6.51	NB
	s166	IgM	V_H558	GRYT	3	Vκ-12	4	0.31	*13*	NB
	s-c6(2)	IgM	V_H558	R	3	Vκ-4	2	0.05	NI	NB
	ps-c7(3)	IgM/G2a	V_H10	DdYVA	3	Vκ-1	2	0.40	*14*	NB
	s-c1(4)	IgG2b	V_H558	EDyYGss	2	Vκ-5	5	0.05	0.12	NB
	s-c2(2)	IgG2a	V_H558	RGRSVY	2	Vκ-1	1	0.04	NI	NB
	s-c3(2)	IgG2a	V_H558	EGWEGGPY	2	Vκ-1	5	0.60	5.78	1
	s-c4(3)	IgG2a	V_H558	GGnYGGS	4	Vκ-21	2	0.04	0.82	NB
	s-c5(3)	IgG2b	V_H558	SrYRg	4	Vκ-1	1	0.47	1.05	NB
	s83	IgG1	V_H558	GYKA	3	Vκ-1	2	0.58	NI	NB
	s2	IgG2a	V_H7183	NLGRRTY	2	Vκ-32	5	7.18	NI	NB
	s5	IgG2b	V_H7183	HRGSLWLRRAD	2	Vκ-19	1	0.34	3.00	4
	s130	IgG1	V_H7183	DLKWLRRG	1	Vκ-19	2	0.27	1.53	<2
	s145	IgG2b	V_HQ52	HKYYDISP	3	Vκ-2	4	0.14	*35%*	NB
111	68	IgM	V_H558	GVARGS	4	Vκ-1	1	EB	EB	NB
	185	IgM	V_H558	DG	3	Vκ-1	1	0.36	*17%*	NB
	c1(4)	IgM/G2a	V_H558	GGSGYD	3	Vκ-8	1	0.91	0.97	NB
	c2(3)	IgG2a	V_H558	GTVIGD	4	Vκ-2	4	0.13	NI	NB
	55	IgG2a	V_H7183	NMATA	3	Vκ-1	2	0.25	*16%*	NB
	67	IgG2a	V_H7183	GSI	1	Vκ-unk	5	2.50	*16%*	4
	33	IgG2a	V_HS107	ASYGSRG	1	Vκ-1	4	1.13	*16%*	<2
163	c4(2)	IgM	V_H7183	KGLRRN	4	Vλ-1	3	1.55	1.09	NB
	42	IgM	V_H7183	RYYGTFL	2	Vκ-Ox1	1	10	1.30	NB
	72	IgM	V_H558	RGITTV	3	Vκ-5	1	NI	1.53	334
	100	IgM	V_H558	RLRWA	3	Vκ-8	2	0.08	NI	NB
	c1(9)	IgG2b/G2a	V_H7183	HYYGSRTY	2	Vκ-8	2	0.28	1.37	1.4
	c2(3)	IgG2a	V_HS107	DpygRTRs	4	Vκ-10	1	0.6	0.62	1
	c3(2)	IgG2a	V_HQ52	KGLRRAG	4	Vλ-2	2	0.5	0.12	1
	47	IgG2b	V_H558	GI	1	Vκ-9	2	0.6	0.17	NB
165	6	IgM	NA[‡‡]	NA[‡‡]	NA[‡‡]	Vκ-23	1	*26%*	3.4	NB
	3m	IgM	V_H558	DPPLRRLYY	4	Vκ-19	1	0.11	NI	<0.025
	5	IgM	V_H558	EGCY	1	Vκ-8	2	NI	NI	2
	33	IgM	V_HQ52	YYYGSPLN	1	Vλ-1	1	EB	NI	<0.20
	41	IgM	V_HQ52	YDGYY	2	Vκ[‡‡]		EB	EB	NB
	52	IgM	V_HQ52	YHSTAPWW	1	NA[‡‡]		EB	NI	<0.25
	54	IgM	V_H36-60	SGRGA	4	Vκ-23	2	0.11	*15%*	NB
	27	IgM	V_H558	DG	3	Vκ-1	1	0.095	NI	NB
	45	IgM	V_H558	EA	1	Vκ-8	2	*17%*	NI	<2

continued

Table 1. *(continued)*

Mouse	Clone[‡]	Isotype	VH			VL		DNA binding specificity[*]		
			V_H[§]	D_H[‖]	J_H	V_L[¶]	J_L	ssDNA	dsDNA	CDLP[**]
	60	IgM	V_H558	GETTVVGKGY	2	Vκ-23	1	NI	8.7	NB
	3	IgG1	V_H558	GDLLWLRRIL	2	Vκ-8	5	0.14	*34%*	40
	14	IgG1	V_H558	RYYGREGY	2	Vκ-10	4	0.16	NI	NB
	49	IgG2a	V_H558	RAWD	1	Vκ-8	1	0.16	NI	NB
202	9	IgM	V_HQ52	YYGSS	4	Vκ-8	2	8.46	*29%*	NB
	17	IgM	V_HQ52	YSDYYGSS	1	Vλ-2	2	NI	NI	NB
	33	IgM	V_H7183	SRWLLRVG	1	Vκ-9	4	NI	5.87	NB
	p38	IgM	V_H7183	QGWDR	4	Vκ-21	1	3.47	NI	NB
	54	IgM	V_H558	LP	1	Vκ-9	2	0.16	*17%*	NB
	61	IgM	V_H558	LIYYYGSI	3	Vκ-Ox1	5	0.29	NI	NB
	80	IgM	V_H558	RGYYGSS	4	Vκ[‡‡]	1	EB	NI	1
	s38	IgM	V_H558	GGRYDL	4	Vκ-1	4	0.22	NI	NB
	135	IgM	V_H558	GYYGSSYS	3	Vκ-8	2	1.97	NI	NB
	105	IgG2a	V_H558	RYYRR	4	Vκ-9	2	2.23	2.12	<2
10	c1(4)	IgG	V_H558	EDrTG	2	Vκ-1	1	0.70	3.17	3.5
25	12m	IgM	V_H558	GRYT	3	Vκ-1	2	0.34	NI	NB
74	c1(2)	IgG	V_H558	EDWDGG	3	Vκ-5	2	0.09	0.14	1
	c2(2)	IgG	V_HS107	DKGRYGA		Vκ-21	1	0.5	*21%*	0.005
83	c1(3)	IgG	V_H7183	GGTR	3	Vκ-19	4	1.91	2.74	<0.05
185	c1(9)	IgG	V_HQ52	NTPLGRRY	2	Vκ-12	1	0.30	0.39	NB

* The DNA binding specificity for ssDNA and dsDNA is presented as the amount (μg/ml) of either ssDNA or dsDNA that was required to produce 50% of maximum binding in the competitive ELISA. Numbers in italics represent values extrapolated from the inhibition curve. Percentages in italics are the maximum percentage inhibition of binding produced by 10 μg/ml competitor. The standard deviation of triplicate wells at each competitor dilution was always ≤10% of the mean OD_{405}. For clones with multiple hybridomas, the data presented are from one representative mAb. NI = no inhibition. EB = enhanced binding; in the presence of the competitor, binding to the solid-phase DNA was increased.

‡ Clones represented by a single hybridoma are designated with a number. Clones with two or more members are designated by a "c" followed by a number. The number of individual hybridomas isolated and analyzed from a clone is in parentheses after each clone number. The "p" and "s" designations of the clones from animal 17 refer to the partial fusion from which the hybridomas were obtained (see text). "p" refers to the first fusion and "s" to the second.

§ Nomenclature according to Brodeur and Riblet (70).

‖ Amino acid sequence for that part of CDR3 contributed by D_H.

¶ Nomenclature according to Potter et al. (71).

** CDLP binding is presented as a ratio obtained by dividing the titer of mAb supernatant that produces 50% of maximum binding to CDLP in the solid-phase ELISA divided by the titer that produces 50% of maximum binding to DNA in the solid-phase ELISA for DNA binding. The dilution of supernatant that produced 50% maximum binding to solid-phase DNA was the same dilution used in each respective competitive ELISA for that supernatant. NB = no binding.

‡‡ The cDNA sequence was not obtained.

germline gene (163-c2, 74-c2, and 111.33; Fig. 1 *D*). If the estimation of preferential V_H gene usage is based on the number of hybridomas rather than the number of clones, preference in V_H gene usage was even stronger ($p \leq 0.004$). Seven hybridomas expressed a V_H homologous to V_H558-BWDNA7 (111-c1 and 111-c2; Fig. 1 *A*, *BWDNA7*). 9–12 hybridomas expressed a V_H homologous to V_HQ52-165.33 (185-c1, 17s.145, and 163-c3; Fig. 1 *C*); and 12 hybridomas expressed a V_H homologous to V_H7183-Vh283 (163-c1 and 83-c1; Fig. 1 *B*, *Vh283*). The latter estimate assumes that the precursors to individual hybridomas are selected independently by antibody receptor–mediated events regardless of the size of the clone of which the respective hybridomas might be members. Although the V_H558 family V_H gene expressed by the anti-DNA hybridoma 3H9 (Fig. 1 *A*, *2F2[3H9]*) was not preferentially expressed among the hybridomas analyzed

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A) Vh558                                                                                                              Clone Size
           1        10        20        30            40           50 abc        60        70        80        90          102
BWDNA16   EIQLQQSGAELVKPGASVKISCKASGYSFT GYNMN WVKQSHGKSLEWIG NINP YYGSTSYNQKFKG KATLTVDKSSSTAYMQLNSLTSEDSAVYYCAR
165.14        -------------------------- ----- -------------- ---- ------------- -------------------------------- RYYGREGY     YFDY    W   1
17s.128   ---------------x-------------- ----- ---L---------- ---- --IT--------- -------------------------------- ALRQGY       ----    -   1
17s-c1    -V------P----------M-------T-- D-Y-- -------------- R--- SN-G--------- ---------L---------------------- EDYYGSS      ---     -   4
165.60    -V------P----------M-------T-- D-Y-- -------------- RV-- SN-G--------- ---------L---------------------- GETTVVGKGY   ----    -   1
163.72    -V------P------D---M-------T-- D-Y-D -------------- Y-Y- NN-G--------- ---------------E-H-------------- RGITTV       ---     -   1
25.12m    QV-----DR------x-----------T-- D---D -------------- Y-Y- NN-G-G------S ---------------E-H-------------- GRYT         -A-     -   1
10-c1     -V------P--------------------- --YVH -----PE----SL- E--- RT-G-T------A -----------------K------------T- EDRTG        ----    -   4
17s.166   -V------P------x-------------- --Y-H -----PE------- E--- ST-GIT------A -----------------K-------------- GRYT         -A-     -   1

2F2       QVQLQQSGPELVKPGASVKISCKASGYAFS SSWMN WVKQRPGKGLEWIG RIYP GDGDTNYNGKFKD KATLTADKSSSTAYMQLSSLTSEDSAVYFCAR
17s-c4    ---------------------------E-- R---- ----G--------- W--- ----IK------G R--------------H-------G-------K GGNYGGS      FYALDY  W   3
17s.13    -----------------------T------ ----- -------------- W-x- R------------ -------I--N--------------T------ SRGYYFGSSRF  F-V     -   1

DNA22     QVQLQQPGAELVKPGASVKLSCKASGYTFT SYWIN WVKQRPGQGLEWIG NIYP GSSSTNYNEKFKS KATLTVDTSSSTAYMQLSSLTSDDSAVYYCAR
111.185   ------------------------------ ----- -------------- ---- ------------- -------------------------------- DG           YAY     W   1
165.27    -------------xxx-------------- ----- -------------- ---- ------------- -------------------------------- DG           ---     -   1
165.49           ----------------------- ----- -------------- ---- ------------- -------------------------------- RAWD         WYFDV   -   1
17s.83    ------------------------------ ----- --M----------- ---- --II-HF-----N -------------------------------G GYKA         WFV-    -   1
163.100   ------------------------------ ---MH -------------- E-N- SNGG--------- -------K--------------E-------TI RLRWA        WF--    -   1
17s-c6    ------------------------------ ---M- -------R------ R-H- SD-E-H--Q---- -------K--T---I----------------- R            F--     -   2
163.47    ------------------------------ N--M- -------R------ R-L- SDTE-H--Q---N -------K-T----IR------E--GI----- GI           YWYFDV  -   1
17s-c3    ------S----AR---------R------- N-D-- --R--A-------- E--- R-GNIY------G -----A-K----T--H--G---G--------- EGWEGGPY     YFD-    -   2

BWDNA7                   VKMSCKASGYTFT SYVMH WVKQKPGQGLEWIG YINP YNDGTNYNEKFKG KATLSSDKSSSTAYMELSSLTSEDSAVYYCAR
111-c1    EVQLQQSGPELVKPGAS------------- ----- -------------- ---- -----K------- ----T--------------------------- GGSGYD       GFAY    W   4
111-c2    ------------------------------ R---- -------------- ---- -----K------- ----T--------------------------- GTVIGD       YYAMD-  -   3
17p.101   ---------------Vx------------- ----- -------------- ---- -----K------- ----T--------------------------S LI           Y-D-    -   1
202.80    ------------------------------ ----- -------------- ---- -----K------- ----T--------------------------- RGYYGSS      YAMD-   -   1
202.S38   ------------------------------ ----- -------------- ---- -----K------- ----T--------------------------- GGRYDL       YYAMD-  -   1
202.135   ------------------------------ ----- -------------- ---- -----K------- ----T--------------------------- GYYGSSYS     W---    -   1
202.61    ------------------------------ ----- -------------- ---- -----K------- ----T--------------------------- LIYYYGSI     ---     -   1
165.3m                       ----------- ----- -------------- ---- -----K------- ----T--------------------------- DPPLRRLYY    YAMD-   -   1

S57(Vh31)             KPGASVKISCKASGYTFT DYYIN WVKQRPGQGLEWIG WIYS GSGNTKYNEKFKD KATLTVDTSSSTAYMQLSSLTSEDSAVYFCAR
17s-c2    QIQLQQSGPELVR----------------- ----- -------------- ---P ------------G -------------------------------- RGRSVY       YFDY    W   2
165.3     ------------R----------------- ----- -------------- ---P ------------G -------------------------------- GDLLWLRRIL   --      -   1
74-c1     Q-R---------R--x-------------- ----- -------------- ---P ------------G -----------------R---------H---K EDWDGG       -V-     -   2
202.105   EV---------------------------- ---MH ----K--------- E--P -----Y------G --S--A-K------------------------ RYYRR        SYAM--  -   1
111.68    -V-----------------L---------- --T-H ----S--------- ---P --------D---G ---M-A-K------------------------ GVARGS       AM--    -   1
165.45    -V-----------------L---------- --T-x ----S--------- ---P --------D---G ---M-A-K--R--------------------- EA           YW---V  -   1

17s-c5    QVQLQQSEAELARPGASVKMSCKASGYTFT RYWMH WVKQRPGQALEWIG AIYP GNSDTNYNQKFKG KAKLTAVTSASTAYMELSSLASEDSAVYYCAR SRYRG        YSMDY   W   3
165.5                       ------------ N---- --------G----- ---- -----S------- --------------------TN--------T- EGCY         YWYFDV  -   1
202.54    -------G----K--------------S-- ----- --------G----- Y-N- SSGY-E------D --T---DK-S-----Q----T----------S LP           WYF-V   -   1

B) Vh7183                                                                                                             Clone Size
           1        10        20        30            40           50 abc        60        70        80        90          102
Vh283     EVMLVESGGGLVKPGGSLKLSCAASGFTFS SYTMS WVRQTPEKRLEWVA TISS GGGNTYYPDSVKG RFTISRDNAKNNVYLQMSSLRSEDTALYYCAR
202.33         ----A-------------------- --G-- -------------- ---G ---------T--- -----------TL------------------- SRWLLRVG     YWYFDV  W   1
163-c1    --K--------------------------- --A-- ------A------- Y--- ---S--------- -----------TL-------------M----S HYYGSRTY     ---Y    -   9
83-c1     --K-M------------------------- --V-- ------A------- Y--- --DS-----NM-- -----------TL-------------M----- GGT          R-AY    -   3
17s.5     ---------A-------------------- D-A-- ------Q------- ---- --IY-----G-Q- -L-------R-TL-------------M----- HRGSLWLRRAD  ---Y    -   1
17s.93    --Q------A-------------------- N-A-- -------------- ---- --SY--------- ---x-------TL------------------- RGTTVY       ---Y    -   1
202.38m   --Q--------------------------- --A-- -------------- A-N- N--S-----T--D -----------TL------------------- QGWDR        AM-Y    -   1

17p.73    EVQLQQSGAELVRPGASVKLSCTASGFNIK DDYMH WVKQRPEQGLEWIG RIDP ANGNTKYAPKFQD KATITADTSSNTAYLQLSSLTSEDTAVYYCAR RDN          YYAMDY  W   1
111.67    ------------------------------ ---L- -------------- ---- ------------- -------------------------------- GSI          WYF-V   -   1

17s.2     EVQLVESGGGLVKPGGSRKLSCAASGFTFS DFGIH WVRQAPEKGLEWVA YISR GSGTIYYADTVKG RFTISRDNDKNILFLQMTSLRSEDTAIYYCAR NLGRRTY      YFDY    W   1
111.55    ------------------------------ -Y-M- -------------- ---S --FN--------- --------A--T--------------M----- NMATA        W-V-    -   1
163.42             --------------------- -Y-M- ------------I- ---G --N---------- ----F---A--T--------------M----- RYYGTFL      ---     -   1
163-c4             --------------------- ---M- -------------- ---S --S---------- --------A--T--------------M----- KGLRRN       YYAM-D  -   2

DNA13     EVKLVESEGGLVQPGSSMKLSCTVFGFSFS DYYMA WVRQFPEKGLEWVA KINY DGSNTYYLDSLKG RFIISRDNAKNILYLQMSSLKSEDTATYFCSR VNYNGLRRS    CFAD    W
17s.130   -----------------------AS--T-- ----- ----V--------- N--- ---S-H------- --------G-------------------Y-T- DLKWLRRG     YWY-DV  -   1

C) VhQ52
165.33    QVQMKESGPDLVQPSQTLSLTCTVSGFSLS SYGVH WFRKPPRKGLEWLG GIW  SGGSIYYTPALSS RLSVSRDISKSQVFFKMSSLQSEDTAVYHCAR YYYGSPLN     WYFDV   W   1
165.41    ---M-----------x------------x- ----- -------------- ---  ------------- -------------------------------- YDGYY        ---Y    -   1
165.52            ---------------------- ----- -------------- ---  ------------- -------------------------------- YHSTAPWW     ---     -   1
202.17    ---MQ------------------------- ----- -------------- ---  ------------- -------------------------------- YSDYYGSS     Y-----  -   1
202.9     ---L-------------------------T ----- -V-Q--G-----V- TMG  W-DKK--NS--K- ---I---T--N---L-L----T----M-Y-TY YYGSS        YYAM-Y  -   1
185-c1    ---L-----G--A---S--I---------T --AIS -V-Q--G------- V--  T--DTS-NS--K- ---I-K-N------L--N---TD---R-Y--- NTPLGRRY     ---Y    -   9
17s.145   ---L-----V--A---S--I---------T --G-H -V-Q--G------- V--  A---TN-NST-M- ---I-K-N------L--N---AD---MFY-VK HKYYDISP     -AF     -   1
163-c3    ---L-Q---G------S--I---------T N-G-H -V-QS-G------- M--  ---NTD-NA-FI- ---I-K-N----------N---AD---I-F--- KGLRRAG      AM-Y    -   2

D) Vh8107
Vh11      EVKLVESGGGLVQPGGSLSLSCAASGFTST DYYMS WVRQPPGKALEWLA LIRNKANGYTTEYSASVKG RFTYSRDNSQSILYLQMNALRAEDSATYYCAR
163-c2    --N--------------------------F- ----N -------------- ------------------- ---I--------------V--T---------- DPYGRTRS     YTMDY   W   3
74-c2     ---------------x------------F- ----- -------------- ---K---D----------- ---I---D------------------------ DKGRYGA      WFA-    -   2
111.33    ----------------------------F- Y---- -----------C-- ------------------- ---L---------------------------T- ASYGSRG      WYF-V   -   1
```

Figure 1. Translated amino acid sequences for VH (one-letter code). The sequences are grouped according to the V_H family from which each VH was derived. Within the VH grouping, sequences are ordered according to homology with the reference sequence. Dashes indicate identity with the reference sequence; blanks indicate either that the VH does not have an amino acid at that position or sequence was not obtained for that region.

in this study, this V_H gene was found to be preferentially expressed among anti-DNA hybridomas from MLR *lpr/lpr* mice (9). There was no apparent preferential expression of a particular J_H or V_H-J_H combination among either IgM or IgG hybridomas.

Arginines in VH CDR3 Derived by Unusual D_H Recombination. More than 50% of the clones, 35 of 63, had a VH with one or more arginines in CDR3 (Table 1 and Fig. 1). This is a highly characteristic feature of anti-DNA antibodies in mice (6, 7, 9, 22–29) and humans (30, 31). This region of the heavy chain is formed by recombination of one or more D_H genes within the 5′ end of a J_H gene and the 3′ end of a V_H gene (32, 33). Arginines are generally rare in CDR3 (9) and are the products of random events in the recombination process (24). As we have demonstrated previously (7), arginines in VH CDR3 can be generated by several different mechanisms. These include N sequence addition (34, 35), such as in 17p.73, 17s.93m, 163.100, and 202.105. A shift from the normal reading frame, RF1 (36, 37), of a D_{FL16} or D_{SP2} gene to a different reading frame, RF3, can also generate arginines in VH CDR3. This frame shift can be caused either by N sequence addition, such as in 163.100 and 165.3 or D_H-D_H recombination (33) or inversion of a D_H gene during recombination, such as in 17s.13. All these mechanisms for D region diversity have also been reported by others (9, 24, 38–40).

Selected V_L Gene Expression among Both IgM and IgG Hybridomas. V_L from each of the $V_\kappa1$, $V_\kappa2$, $V_\kappa4$, $V_\kappa5$, $V_\kappa8$, $V_\kappa9$, $V_\kappa10$, $V_\kappa12$, $V_\kappa19$, $V_\kappa20$, $V_\kappa21$, and $V_\kappa23$ gene families and from the V_κOx-1, V_κRF, $V\lambda1$, and $V\lambda2$ germline V_L genes were expressed among the hybridomas analyzed in this study (Fig. 2). The estimated number of different germline V_L genes that could be used to generate an anti-DNA antibody is 62 (see Materials and Methods). Based upon this number of V_L genes, the probability that three clones would express the same V_L gene is <0.018. Therefore, there was preferential usage of a V_L gene derived from each of the $V_\kappa2$ and $V_\kappa5$ germline families. The preferentially used $V_\kappa2$ gene was 92% homologous to the $V_\kappa2$ gene expressed by the previously identified anti-DNA hybridoma BXW-DNA14 (17p.3, 111-c2, and 17s.145; Fig. 2 *C*) (22). The preferentially used $V_\kappa5$ gene was 92% homologous to the $V_\kappa5$ gene expressed by the anti-DNA hybridoma MRL-DNA22 (163.72, 17s-c1, and 74-c1; Fig. 2 *D*) (22). Preference for V_L derived from the $V_\kappa1$ and $V_\kappa8$ families was much stronger ($p \leq 0.000080$) for seven or more clones expressing the same (95% homologous) $V_\kappa1$ or $V_\kappa8$ gene. The $V_\kappa1$ genes expressed by all the clones in Fig. 2 *A* are ≥91% homologous to the $V_\kappa1$ expressed by the previously reported anti-DNA hybridoma MRL-DNA4 (22). Most of the clones expressing a V_κ gene from the $V_\kappa8$ group expressed a $V_\kappa8$ gene ≥98% homologous to the V_κ gene expressed by the anti-DNA hybridoma DNA5 from clone 4 (7).

The preference for $V_\kappa1$ and $V_\kappa8$ was apparent among both IgM and IgG hybridomas ($p \leq 0.0027$ and $p < 0.00013$, respectively). Among the IgM clones, five clones (17s.93m, 17p.101, 25.12, 111.185, and 165.27; Fig. 2 *A*) each expressed a VL derived from the same $V_\kappa1$ gene, and three clones (202.9, 165.45, 111-c1, and 165.5; Fig. 2 *B*) each expressed a VL derived from the same $V_\kappa8$ germline gene. Among IgG clones, four expressed a VL derived from the same $V_\kappa1$ germline gene (17s-c2, 17s-c3, 17s-c5, and 17s.13; Fig. 2 *A*), and four expressed a V_L derived from the same $V_\kappa8$ germline gene (165.49, 163-c1, 111-c1, and 165.3; Fig. 2 *B*). These results indicate preferential clonal selection and expansion of anti-DNA B cells expressing either $V_\kappa1$ or $V_\kappa8$ during both the early IgM and late IgG anti-DNA response in the autoimmune mice from which our panel of hybridomas was obtained.

$J_\kappa1$ Junctional Diversity among VL of Anti-DNA Hybridomas. Assuming that each J_κ has an equal probability for expression among anti-DNA B cell clones, $J_\kappa1$ was overrepresented and $J_\kappa4$ and $J_\kappa5$ were underrepresented among the clones listed in Table 1 ($p < 0.025$). This unequal representation of $J_\kappa1$ is consistent with normal J_κ usage in mice (41). However, 8 of the 23 $J_\kappa1$ clones had arginine (CGG) as the first codon encoded by $J_\kappa1$ instead of the germline tryptophan (TGG). This position would correspond to position number 96 in VL CDR3, according to the numbering convention of Kabat et al. (42). This conversion would most likely occur from junctional diversity between V_κ and J_κ (43) but could also be due to somatic mutation. 17 hybridomas from eight clones expressed the altered $J_\kappa1$ (IgM clones 111.185, 17p.3, and 163.72; and IgG clones 10-c1, 111-c1, 163-c2, 17s.5, and 74-c2). Each of the IgG clones had two to nine members, indicating that B cells with VL derived from $J_\kappa1$ with the tryptophan→arginine conversion may have been selectively expanded.

Selection for V_H558-$V_\kappa1$ and V_H558-$V_\kappa8$. Assuming an equal probability for any of the possible anti-DNA V_H genes to be expressed with any of the possible V_L genes in a given B cell, significantly more clones expressed V_H-V_L combinations generated from a V_H558 family V_H and either a $V_\kappa1$ or $V_\kappa8$ family V_L than would have been expected if V_H and V_L genes assorted randomly ($p < 0.0018$ for V_H558-$V_\kappa1$, and $p < 0.029$ for V_H558-$V_\kappa8$). When the data were analyzed to determine if a particular V_H gene-V_L gene combination was expressed at a higher-than-expected frequency among the clones, one combination was found to be preferentially used. The IgG hybridoma 17.s83 and the IgM hybridomas 111.185 and 165.27 expressed nearly identical V_H and V_L genes. The V_H gene was similar (≥93% homology) to the V_H558 family V_H gene previously reported for the anti-DNA hybridoma BXW-

An "x" indicates sequence ambiguity for the relevant position. The sequences are numbered according to Kabat et al. (42); CDR regions are enclosed within rectangular boxes. The clone numbers of IgM-producing hybridomas are underlined. The nucleotide sequences from which these translated sequences were derived have been submitted to the EMBL/GenBank Sequences Libraries. The origin of the reference sequences are (*A*) the V_H558 family V_H genes for the anti-DNA hybridomas BXW-DNA16, MRL-DNA22, and BXW-DNA7 (22) and the V_H for the anti-DNA hybridomas 2F2 and S57 (Vh31) (9); (*B*) the V_H7183 family germline gene Vh283 (9, 72) and the anti-DNA hybridoma DNA13 (7); (*D*) the V_HS107 family germline gene Vh11 (73).

	1 10 20	30	40	50	60 70 80	90		Clone Size
A) Vκ1								
MRLDNA4	DVVMTQIPLSLPVSLGDQASISC	RSSQSLVHS NGNTYLH	WYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	SQSTHVPYT	F	
17s-c2	------T----------------	--------- -------	---------------	-------	--------------------------------	-------W-	-	2
17s-c3	------T----------------	--------- -------	---------------	-------	--------------------------------	-------L-	-	3
17s-c5	------T----------------	--------- -------	---------------	-------	--------------------------------	-------W-	-	1
17s.93	------T----------------	--------- -------	---------------	-------	--------------------------------	-------L-	-	1
17s.13	------T----------------	--------N -------	---------------	-------	--------------------------------	--C----W-	-	1
17p.101	------T----------------	--------N ------D	---------------	Rx-----	--------------------------------	F-G----Q-	-	1
111.55	-----------T-----------	--------N ------Y	---------------	R------	--------------------------------	L-G----F-	-	1
25.12	-----------------------	--------- ------Y	---------------	R------	--------------------------------	L-G------	-	1
111.185	------T----------------	--------- ------Y	---------------	R------	--------------------------------	F-G--I-R-	-	1
165.27	---------x-----	--------- ------Y	---------------	R------	--------------------------------	FxG----W-	-	1
17s.83	----A-T----------------	--------- ------Y	-F-------------	RT-----	--------------------------------	F-G------	-	1
17sp-c7	-T----T----------------	------EN- ------N	----------Q----	R------	--------------------------------	L-V----Y-	-	3
202.s38	------T----------------	-----I--- ------E	---------------	-------	------------------------------Y-	F-GS---F-	-	1
111.68	------T----------------	-----I--- ------E	---------------	-------	------------------------------Y-	F-GS---W-	-	1
111.33	--L---T----------------	-----I-NR ------E	---------------	-------	------------------------------Y-	F-GS-x-F-	-	1
10-c1	------------------V----	--------D --D---Y	---------------	-------	---------------------L----------	--G-Y--R-	-	4
B) Vκ8								
DNA5	DIVMSQSPSSLAVSAGEKVTMSC	KSSQSLLNSRTRKNYLA	WYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC	KQSYNL RT	F	
202.9	-----------------------	-----------------	---------------	-------	--------------------------------	----H-	-	1
165.45	-----------------------	-----------------	---------------	-------	--------------------------------	------ Y-	-	1
165.49	.....................--	-----------------	---------------	-------	--------------------------------	-----x W-	-	1
163-c1	-----------------------	-----------------	---------------	-------	--------------------------------	------ Y-	-	9
111-c1	-----------------------	-------K---------	---------------	-------	----------------------------I---	------ --	-	4
165.3	-----------------------	--------R--------	---------------	-------	--------------------------------	------ L-	-	1
165.5	-----------------------	--M--------------	---------------	-------	--------------------------------	------ Y-	-	1
163.100	----T-------M-V-Q------	---------SNQ-----	--------------V	F------	------I---------------------D-F-	Q-H-STPY-	-	1
202.135	N-M-T------------------	-----V-Y-SNQ-----	---------------	-------	--------------------------------	H-YLSS Y-	-	1
C) Vκ2								
BWDNA14	DVVMTQTPLTLSVTIGQPASISC	KSSQSLLDS DGKTYLN	WLLQRPGQSPKRLIY	LVSKLES	GIPDRFTGSGSGTDFTLKISRVEAEDLGVYYC	WQGTHFPRT	F	
17p.3	-----------------------	-------H- N------	-----------L---	-------	-V----S-------------------------	L-A------	-	1
111-c2	---------S-------------	-------H- N------	-----------L---	-------	------S---------------V---------	L-H----F-	-	3
17s.145	---------S-------------	R------H- ------x	-----------L---	-------	------S---------------V---------	L-H--C-x-	-	1
D) Vκ4/5								
DNA22	QIVLTQSPAIMSASLGERVTMTC	SASSS VSSSYLY	WYQQKPGSSPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQYSGYPFT	F	
163.72	--------------L--------	T---- ------H	---------------	-------	--------------------------------	H--HRS-R-	-	1
17s-c1	--------------L--------	T---- -R----H	---------------	-------	--------------------------------	H--HRS-L-	-	4
74-c1	----I---------L--------	T---- -R----H	---------------	-------	---------------A----N-----------	H-DHRS-P-	-	2
17s-c6	--------------P--K--L--	----- -----LY	---------------	X------	----------------------------S-FC	H-W-S--Y-	-	2
E) Vκ9								
Vk-41	DIQMTQSPSSLSASLGERVSLTC	RASQ DIGSSLN	WLQQEPDGTIKRLIY	ATSSLDS	GVPKRFSGSRSGSDYSLTISSLESEDFVDYYC	LQYASSP		
202.105	-----------------------	---- E-SGY-S	----K----------	S--T-N-	---------------------------A----	-------YT	F	1
Vk9.42	DIQMTQSPSSLSASLGGKVTITC	KASQ DINKYIA	WYQHKPGKGPRLLIH	YTSTLQP	GIPSRFSGSGSGRDYSFSISNLEPEDIATYYC	LQYDNL		
202.33	-----------------------	---- --x----	---------------	-------	---------------x----------------	-----x F-	-	1
202.54	-----------------------	---- -------	---------------	-------	--------------------------------	------ Y-	-	1
DNA13	DIKMTQSPSPMYASLGERVTITC	KASQ DINNYLS	WFQQKPGKSPKTLIY	RADRLVD	GVPSRFSGSGSGQDYSLTISSLEYEDMGIYYC	LQYDEFPYT	F	
163.47	---------S-----E-------	---- -------	----I----------	--K----	--------------------------------	-H--- --	-	1

Figure 2. Translated amino acid sequences for VL. The organization of the sequences is as in Fig. 1 according to the V_κ subgroups as defined by Potter et al. (71) and numbering according to Kabat et al. (42). The nucleotide sequences from which these translated sequences were derived have been submitted to the EMBL/GenBank Sequences Libraries. The sources of the reference sequences are (*A*, *C*, and *D*), respectively, V_κ from anti-DNA mAbs MRL-DNA4, BXW-DNA14, and MRL-DNA22 (22); (*B* and *E*), respectively, V_κ genes from the anti-DNA mAbs DNA5 and DNA13

DNA22 (Fig. 1 *A*, *BWDNA22*) (22). The V_κ gene was the same (≥98% homology) V_κ1 family gene previously reported for MRL-DNA4 (Fig. 2 *A*, *MRLDNA4*). The probability that this V_H-V_L combination would have appeared three times by chance among the 63 clones is <0.0002. These results are even more striking since each of the three clones that express the V_H558-*BWDNA22*-V_κ1-*MRLDNA4* combination was obtained from a different animal. Moreover, 111.185 and 165.27 are nearly identical in both VH and VL, including the D_H-encoded VH CDR3. These two hybridomas differed in their V_H and V_L sequences by only two identifiable amino acids at positions 94 and 96 in VL CDR3 (Fig. 2 *A*). The difference in position 96 between the two VL sequences is due to the J_κ1 difference discussed above. 165.27 has the germline tryptophan at this position, while 111.185 has arginine.

IgM and IgG Anti-DNA Are Structurally Similar. The data in Table 1 and Figs. 1 and 2 indicate that the IgM and IgG populations are completely overlapping with respect to VH and VL structure. The clones 17s.83, 111.185, and 165.27 described above demonstrate particularly well the similarity in structure between IgM and IgG anti-DNA. In two clones from two different mice, both IgM and IgG hybridomas were obtained from the same clone (Fig. 3). The 17ps-c7 clone is particularly interesting. The hybridomas from this mouse are divided into p and s groups. The p group was generated

	1 10 20	30	40	50	60 70 80	90		Clone Size
F) Vκ10								
NC6-C8	DIQMNQSPSSLSASLGDTITITC	HASQ NINIWLN	WYQQKPGNIPKLLIY	KASNLHT	GVPSRFSGSGSGTGFTLTISxLQPEDIATYYC	QQGQSYPYT	F	
165.14	-----------------------	R--- ------S	---------------	-------	-------------D------S-----------	L------L-	-	1
163-c2	------------------V----	R--- ------S	---------------	-T-----	-------------D------S-----------	L---T--R-	-	3
G) Vκ12								
k2	DIQMTQSPASLSASVGETVTITC	RASG NIHNYLA	WYQQKQGKSPQLLVY	NAKTLAD	GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC	QHFWGTPP		
<u>S17.166</u>	----------------x------	---E --YS---	---------------	------E	--------------F-----------------	--HYN-LIFT	-	1
185-c1	-----------------------	---E --YS---	---------------	------E	--------------F-----------------	--HY----T	F	9
H) Vκ19								
AM17-26	NIVMTQSPKSMSMSVGERVTLSC	KASE NVGTYVS	WYQQKPEQSPKLLIY	GASNRYT	GVPDRFTGSGSATDFTLTISSVQSEDLADYFC	GQSYSYPLT	F	
17s.5	-----------------------	---- -------	-----S---------	-------	-----------------------A------H-	--x--x-R-	-	1
<u>165.3m</u>	......-----------------	---- -------	------x---Q----	-------	-----------------------A------H-	-------F-	-	1
83-c1	D------Q-F--T---D--SVT-	---Q -A-NN-A	------G----A---	S--Y--S	-----------G-------NN-------E---	Q-YNN----	-	3
17s.130	D------H-F--T---D--SIT-	---Q D-NAA-A	------G--------	STAY-C-	-----------G----F------A----V-Y-	Q-H-NT-P-	-	1
I) Vκ20								
C8.5	ETTVTQSPASLSMAIGEKVTIRC	ITST DIDDDMN	WYQQKPGEPPKLLIS	EGNTLRP	GVPSRFSSSGYGTDFVFTIENMLSEDVADYYC	LQSDNLPLT	F	
17s.2	------------V-T----AL--	---- -------	---------------	-------	---------------------T----------	--T--M---	-	1
J) Vκ21								
AM15	DIVMTQAAPSVPVTPGESVSVSC	RSSKSLLHS NGNTYLY	WFLQRPGQSPQLLIY	RMSNLAS	GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC	MQHLEYP		
<u>17s.128</u>	-L-----------------I--	---T----- S-xHR--	---------------	x------	----------x--D-----------F-----	--S----YT	F	1
VK-21E	DIVLTQSPASLAVSLGQRATISC	RASKSVST SGYSYMH	WYQQKPGQPPKLLIY	LASNLES	GVPARFSGSGSGTDFTLNIHPVEEEDAATYYC	QHSRELP		
74-c2	-----------T-----------	-T------ P--T---	---------------	-------	----------------------------S---	--T--H-R-	F	2
17s-c4	-----------------------	---Q---- -A---I-	--------------K	F------	---------------------------T----	---W-I-F-	-	3
<u>17p.73</u>	N----------------------	---E--DS Y-N-F--	---------------	-------	-----------R-----T-D---AD-------	-QNN-D-WT	-	1
<u>202.38m</u>	-----------------------	---E--DN Y-I-F-N	-F-------------	A---QG-	---------------S-----M--D-T-M-F-	-Q-K-V-P-	-	1
K) Vκ23								
Dp12	divltqspatLSVTPGDRVSLSC	RASQ SISNYLH	WYQQKSHESPRLLIK	YASQSIS	GIPSRFSGSGSGTDFTLSINSVETEDFGMYFC	QQSNSWPYT	F	
<u>165.60</u>		--N--	---------------	-------	--------------------------------	-------Q-	-	1
<u>165.6</u>		N--	------D--------	---K---	--------------------------------	-------Q-	-	1
<u>165.54</u>	---S--E---F--	---- --GTSI-	----RTNG-------	---E---	----------------------S--IAD-Y-	-------x-	-	1
L) VκRF								
MRLRF24	DVQITQSPSYLAASPGETITINC	RASK SISKYLA	WYQEKPGKTIKLLIY	SGSTLQS	GIPSRFSGSGSRTDFTLTISSLEPEDFAMYYC	QQHNKYPLT	F	
111.67	-----------------------	---- -------	---------------	-------	--------------------------------	---------	-	1
M) VκOx-1								
45.21.1	QIVLTQSPAIMSASPGEKVTMTC	SAS SSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSNPLT	F	
<u>202.61</u>	-----------------------	--- -------	---------------	-------	--------------------------------	---------	-	1
<u>163.42</u>	--------------------IS-	--- ------Y	-----P-S---P--F	R--N---	--------------------------------	--YH-Y-W-	-	1
N) Vλ1/2								
Vλ-2	QAVVTQESA LTTSPGGTVILTC	RSSTGAV TTSNYAN	WVQEKPDHLFTGLIG	GTSNRAP	GVPVRFSGSLIGDKAALTITGAQTEDDAMYFC	ALWYRNHFV	F	
163-c3	--------- -------------	------- -------	---------------	-------	--------------------------------	----ST-Y-	-	2
<u>202.17</u>	--------- -------------	------- -------	---------------	-------	--------------------------------	----ST-Y-	-	1
<u>163-c4</u>	--------- ------E--T---	C------ -------	---------------	--N--G-	---A-------------------E-I---	----S---I	-	2
<u>165.33</u>	--------- ------E--T---	------- -------	---------------	--N----	---A-------------------E-I---	----S-xW-	-	1
Vλ-1	--------- ------E--T---	------- -------	---------------	--N----	---A-------------------E-I---	----S--		

(7); (*E*) the $V_\kappa 9$ germline gene vk41 (74) and V_κ 9.42 from hybridoma 15-56-1 (75); (*F*) V_κ from the NC6-C8 hybridoma (76); (*G*) the $V_\kappa 12$ germline gene k2 (77); (*H* and *J*), respectively, V_κ from the AM17-26 and AM15 rheumatoid factor hybridomas (49); (*I*) V_κ from the anti-DNA hybridoma C8.5 (58); (*J*) the germline $V_\kappa 21E$ gene (78); (*K*) V_κ from the anti-DNA hybridoma Dp12 (9); (*L*) V_κ from the rheumatoid factor hybridoma MRL-RF24 (79); (*M*) V_κ from the antidextran hybridoma 45.21.1 (80); and (*N*) the germline $V_\lambda 1$ (81) and $V_\lambda 2$ genes (82).

from a partial splenectomy of this mouse at the age of 24 wk, when the mouse's serum IgM anti-DNA titer was 1,800 and IgG anti-DNA titer <90. Five IgM anti-DNA hybridomas out of a total of five were analyzed from this fusion (Table 1). The remaining half of the spleen was removed 1.5 mo later when the mouse's serum IgG anti-DNA titer was >2,400. Six IgM and 20 IgG hybridomas out of a total of 94 were analyzed from this fusion. The IgG hybridoma 17s.161 derived from the second fusion was found to be clonally related to IgM hybridomas 17p.79 and 17p.80 obtained from the first fusion (Fig. 3 *A*). Clonality of these hybridomas was confirmed by identity of both productive and nonproductive J_H and J_κ rearrangements (21) (not shown). The IgM hybridoma 111.19 and the IgG hybridomas 111.61, 111.100, and 111.109 from clone 111-c1 were also found to be clonally related, although these hybridomas were all generated in the same fusion (Fig. 3 *B*). Somatic mutations were apparent in both clones. The effects of these mutations on specificity for DNA will be discussed elsewhere (Jou et al., manuscript in preparation). These results indicate that some, if not all, of the secondary, IgG anti-DNA antibodies are generated by selective clonal expansion of B cells that are initially stimulated to generate the primary IgM anti-DNA antibodies in autoimmune (NZB × NZW)F_1 mice.

Structural Basis for ssDNA vs. dsDNA Specificity and Selection for dsDNA Specificity among Clonally Expanded B Cells.

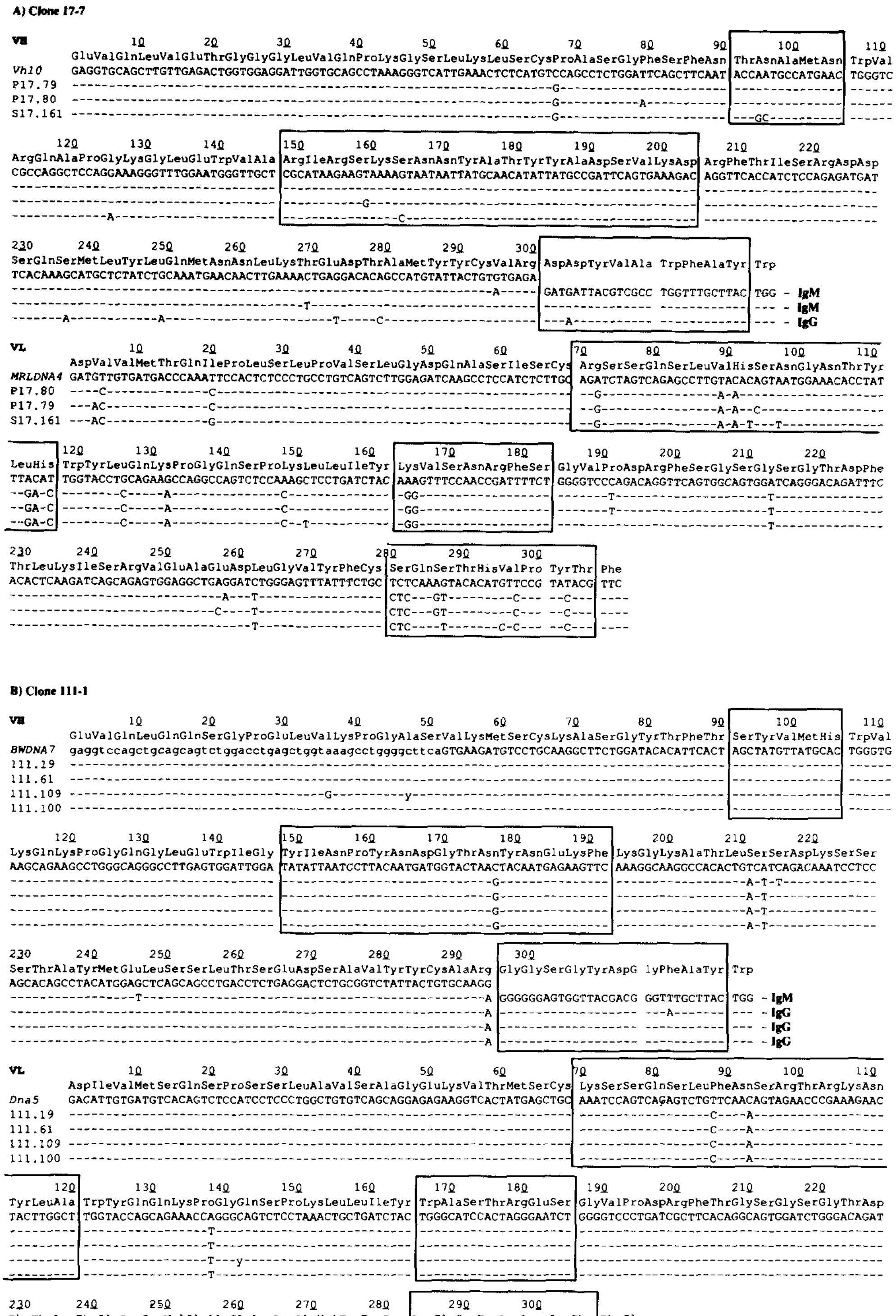

Figure 3. VH and VL cDNA sequences for the mAbs from (*A*) clone 17ps-c7 and (*B*) clone 111-c1. The reference sequences are (*A*) for V_H, Vh10 (83), and for V_L, the V_κ from MRL-DNA4 (22); and (*B*) for V_H, V_H from BXW-DNA7 (22), and for V_L, V_κ from DNA5 (7). Dashes indicate identity of nucleotides with the reference sequences. CDR regions are enclosed within boxes. The Ig H isotype of the respective mAb is indicated. Numbering is by sequential nucleotides.

The mAb produced by each hybridoma was assayed for specificity of binding to ssDNA, dsDNA, and cardiolipin (CDLP)[1] (Table 1). Although all the mAbs bound to DNA in a direct ELISA (Materials and Methods), a small number (8 IgM mAbs out of 107 total mAbs) were not competitively inhibited by either ssDNA or dsDNA in the competitive ELISA. This may be a reflection of the higher avidity of these antibodies for binding to immobilized DNA. In fact, six of the IgM mAbs (17p.3, 111.68, 165.33, 165.41, 165.52, and 202.80) that were not competitively inhibited demonstrated increased binding to the solid-phase DNA in the presence of competitor DNA (Table 1). The most likely explanation for this observation is that these antibodies have relatively low affinity for binding to DNA. In the presence of competitor and because of the high avidity of IgM, the antibodies are able to form a lattice that stabilizes their binding to the immobilized DNA. There was no significant relationship between particular V_H or V_L gene expression by different hybridomas and the absolute ability of the antibodies from those clones to bind to ssDNA vs. dsDNA or CDLP ($0.05 < p < 0.10$). Likewise, there was no correlation between arginines in VH CDR3 and specificity for DNA among the antibodies. However, as discussed below, the position of arginines in VH CDR3 had a remarkable effect on the DNA specificity of the respective mAb; and particular VH and VL structural combinations also had demonstrable effects on DNA specificity.

All of the mAbs that had an arginine at position 96 in VL bound to dsDNA. As indicated above, arginine at this position was most likely derived from junctional diversity in V_κ to $J_\kappa 1$ recombination. The recurrent nature of this randomly generated structure among clones using $J_\kappa 1$ suggests that this structure may have been specifically selected. This possibility is supported by results from a comparison of mAbs 111.185 and 165.27. Although both mAbs bind to ssDNA very well (Table 1), 111.185 binds poorly but measurably and consistently to dsDNA. These two antibodies differ in VH and VL at only two identifiable positions. 111.185 has an isoleucine instead of valine at position 94 in VL CDR3. More importantly, 111.185 has an arginine instead of tryptophan at position 96 in VL CDR3.

There was a very interesting and highly significant trend relative to the specificity of antibodies produced by IgM vs. IgG clones. A significantly higher percentage of IgG clones produced antibody that bound to both ss- and dsDNA or dsDNA alone as opposed to ssDNA binding alone (Table 1). Of 27 IgG clones, 21 had one or more hybridomas that produced anti-dsDNA antibody, compared with 12 of 34 IgM clones ($p < 0.001$). This difference was even more pronounced when individual hybridomas rather than clones were compared ($p < 0.0005$). The difference in dsDNA binding between IgM and IgG mAbs was also observed within a single clone, 17ps-c7. The IgM mAbs 17p.79 and 17p.80 both bound to ssDNA with relatively high avidity but did not bind to dsDNA (Jou et al., manuscript in preparation). The IgG mAb from the clonally related hybridoma 17s.161, obtained 1.5 mo later, bound to ssDNA and dsDNA.

Discussion

The results from our analyses of the hybridomas described above confirm our previously reported results that the IgG anti-DNA hybridomas from an individual (NZB × NZW)-F_1 mouse were oligoclonal and somatically mutated (6, 7), and therefore had the characteristics of secondary immune antibodies (21, 44–48). The present results indicate that the oligoclonality of IgG anti-DNA hybridomas observed previously was not unique to a single mouse and may, in fact, be a general characteristic of IgG anti-DNA hybridomas obtained from individual (NZB × NZW)F_1 mice. These results corroborate the hypothesis that the spontaneous IgG anti-DNA antibody response characteristic of these mice is generated as a clonally selective, Ig receptor–specific immune response. IgG anti-DNA hybridomas from MRL *lpr/lpr* mice are also oligoclonal and the mAbs from those hybridomas are characteristic of secondary immune antibodies (8, 9, 25). Oligoclonality of IgG anti-DNA antibodies has also been observed among anti-DNA mAbs from autoimmune (NZB × SWR)F_1 mice (27) and among $V_H 11$ encoded anti-DNA in (NZB × NZW)F_1 mice (28).

The IgM anti-DNA hybridomas from the mice in this study also appear to have been derived from B cells that were stimulated in a clonally selective, antigen-specific manner. This conclusion is based on the statistical analyses of V_H and V_L gene expression among the IgM clones. Even though each of the 44 V_H and 62 V_L germline genes in the estimated anti-DNA V gene repertoire could encode an anti-DNA antibody, two V_H and two V_κ genes were each expressed in a much larger number of IgM hybridomas than would have been expected in the absence of antibody receptor–mediated selection. The preferential V gene expression among IgM hybridomas was not the end result of the process by which hybridomas were chosen for cloning since the only criterion used was the ability of the antibody in the respective fusion well to bind DNA. Based upon the results from the competitive ELISA used to test the specificity of each mAb (Table 1), the selection of hybridomas for cloning was not biased with respect to the specificity or affinity of the respective mAbs. As the results in Table 1 clearly demonstrate, hybridomas producing both high- and low-affinity mAb were represented in our sample. We (4) and others (49) have demonstrated that the hybridomas obtained from autoimmune mice are representative of the serum antibody in the fusion donor.

This report provides the first demonstration that both IgM and IgG anti-DNA antibodies within individual autoimmune mice may be derived from the same B cell clones. The results demonstrate that the V regions of IgM and IgG anti-DNA antibody in autoimmune (NZB × NZW)F_1 mice are structurally similar. Also, the V_H and V_L genes that were preferentially expressed among the IgM anti-DNA hybridomas were also preferentially expressed among the IgG hybridomas. Within individual mice, both IgM and IgG anti-DNA antibodies may be produced by clonally related B cells. Therefore, B cells that are selectively stimulated to produce the IgM anti-DNA seen early in autoimmune (NZB × NZW)F_1 mice may differentiate and clonally expand to generate the IgG anti-DNA seen later in the same mice. In addition, the results demonstrate that there was greater specificity for dsDNA binding among the IgG mAbs than the IgM. This may indicate preferential selection for dsDNA specificity among B cell clones that are stimulated to expand and differentiate to IgG production. The results suggest that the stimulus for this B cell clonal expansion may be native DNA. The results are also consistent with and may explain the observed progression of anti-DNA autoantibody in both mouse and human lupus from more ssDNA-specific to more dsDNA-specific antibody (1, 3). Taki et al. (50) have likewise isolated an IgM hybridoma from an (NZB × NZW)F_1 mouse that may be clonally related to an IgG hybridoma from the same mouse. The IgG mAb had much higher avidity for dsDNA than the IgM mAb. The VH used by both hybridomas was the same, and H chain rearrangements in the two hybridomas were the same. However, since the authors did not analyze the respective hybridomas for either L chain sequences or rearrangements, the clonal relatedness of the two hybridomas cannot be assured.

Results from previous studies of B cell activity and antibody production in autoimmune (NZB × NZW)F_1 mice led to the hypothesis that anti-DNA antibody was a byproduct of polyclonal B cell activation (reviewed in reference 14). In light of the results from analyses of the effect of the host environment in which B cells develop on the ontogeny of anti-DNA autoantibody (15, 51) and the clonotypic analyses of IgG anti-DNA antibodies in autoimmune mice (6, 8), this hypothesis has recently been modified to propose that the early, IgM anti-DNA B cells are polyclonally activated and that antigen-specific (DNA) selection occurs subsequent to this event (14). This process would yield IgM anti-DNA with the structural characteristics of a randomly selected population of DNA-specific antibodies and IgG anti-DNA with the structural characteristics of a clonally selected population of antibodies. Our results are not consistent with this hypothesis. Although the results cannot exclude the existence of a population of polyclonally activated B cells in the (NZB × NZW)F_1 mice used in this study, they suggest that both the IgM and IgG anti-DNA hybridomas were derived from B cells stimulated by antibody receptor–specific events. As discussed below, those events were probably antigen-specific stimulation, most likely by DNA or complexes containing DNA.

Previous analyses of V gene diversity among autoimmune anti-DNA antibodies have demonstrated preferential expression of V_H genes from the V_H558 family (9, 25, 52). Radic et al. (25) estimated that 15 V_H558 genes may encode anti-DNA (95% C1 = 9, 42). Using the identical pair method (18), we estimated the number of V_H558 genes that could encode anti-DNA to be 24 (95% CI = 15, 36), which agrees reasonably well with the previous estimate. The recurrence of particular V_H genes among the anti-DNA hybridomas analyzed in this study is striking particularly when compared with the V_H gene expression seen among anti-DNA hybridomas in previously published studies. A summary of the recurrence of V_H and V_L genes among all previously published spontaneous anti-DNA antibody variable region sequences is presented in Tables 2 and 3. The V_H genes that were preferentially expressed among the hybridomas sequenced in this study have been recurrent among the anti-DNA antibodies sequenced in other laboratories. Moreover, even the V_H genes that were not preferentially expressed among our population of hybridomas are recurrent among the total murine population of anti-DNA antibodies for which VH sequences have been published (Table 2). Preferential expression of V_L genes that has not previously been apparent among anti-DNA (9, 25) could be identified here, because of the larger number of sequences analyzed in this study (Fig. 2). Those V_L genes that are preferentially expressed among the hybridomas in this study are recurrent among the total population of mouse spontaneous anti-DNA for which VL sequences have been reported (Table 3). Like the V_H genes, even those V_L genes that were not preferentially expressed among our population of hybridomas are recurrent in the total population of mouse spontaneous anti-DNA. The V_H558-V_κ1 and V_H558-V_κ8 combinations that were preferentially expressed among the hybridomas analyzed in our studies have also been observed among previously analyzed anti-DNA hybridomas (7–9, 24).

The recurrent nature of particular V_H-V_L combinations and individual V_H and V_L genes, as described above, is indicative of a strong preference for these variable region structures among anti-DNA hybridomas. The rather large number of V_H and V_L genes that can potentially generate an anti-DNA antibody indicates that there is no V_H or V_L gene restriction for the generation of these antibodies. Even the most highly represented V_H and V_L genes were found in only 13% and 11% of the clones, respectively. Rather, the recurrent and preferential use of particular V_H-V_L combinations is more consistent with clonal selection of anti-DNA B cells through antibody receptor–mediated events. As discussed below, the most likely event to control such selection is antigen-specific selection by DNA or DNA complexes. The lack of V_H and V_L restriction is consistent with results from previous studies of V_H gene diversity among anti-DNA hybridomas from an individual (NZB × NZW)F_1 mouse (10) and from MRL *lpr/lpr* mice (9). Therefore, as previously discussed in detail (7, 9), shared idiotypy among anti-DNA (5, 53–57) is unlikely to result from V_H or V_L gene restriction. Rather, shared idiotypy among anti-DNA is more likely related to structures that control the specificity of anti-DNA (9).

All the clones with the junctionally derived arginine in J_κ1 produce anti-DNA antibodies that bind dsDNA (Table 1, and Jou et al., manuscript in preparation). Moreover, this same J_κ1 arginine has appeared in the anti-DNA hybridoma DNA4 and the clonally related hybridomas DNA5, DNA6, and DNA7 from our laboratory, and among anti-DNA hybridomas from other laboratories: B62 (29) and BxW-DNA14 (22) from (NZB × NZW)F_1; 13 and 30 from (SWR × NZB)F_1 no. 7 (27); and 2B11 from BALB/c (58). All the monoclonal anti-DNA with J_κ1 that have arginine at position 96 bind dsDNA. These results suggest that arginine at position 96 in VL CDR3 may be a highly selected structure, especially for dsDNA binding. The canonical L chain for Id^{CR1} antiarsonate antibodies also has an arginine at position 96 in VL CDR3 that appears to be generated from junctional diversity (59). This VL structure is highly selected among antiarsonate antibodies. Arginines in both VH CDR3 and position 96 VL CDR3 are generated by random, non-template-encoded processes (7, 9, 24, 25, 29) and are relatively rare among antibodies in general (9). Therefore, there

Table 2. *Recurrent V_H Gene Usage among Spontaneous Anti-DNA Autoantibodies*

V_H gene group*	Relevant clones from Fig. 1‡	Hybridomas or clones from other laboratories§	Reference
V_H588-BWDNA16	165.14, 17s.128	BXW-DNA16	22
		A52	24
		C72	29
		BV16-19	26
	17s-c1, 165.60	Clones H and I, 82-3	25
		H130	23
		A6.1	67
-2F2(3H9)	17s.c4, 17s.13	Clones A, B, and E; D20	9
		Clone 4	7
-MRLDNA22	111.185, 165.27, 165.49, 17s.83	MRL-DNA10, MRL-DNA22	22
	163.100	564, 550, 567, 563 05, 11, 12	27
-BWDNA7	111-c1, 111-c2, 17p.101	BXW-DNA7	22
	202.80, 202.s38, 202.135	8-1, D30	25
	202.61, 165.3m	D444, D44	24
		BWR4	29
-S57(Vh31)	17s-c2, 165.3, 74-c1	Clones C and F	9
V_H7183-Vh283	163-c1, 83-c1	Clone D, DP12, S106	9
		33-2	25
-DNA13	17.s130	Clone 3, DNA3, DNA4	7
V_HS107-Vh11	163-c2, 74-c2, 111.33	D42	24
		B62	29
		DP1	9
		Clones 1–7	28
V_HQ52	163-c3	D23	84
		9-15, 9-4	50
V_H10	17ps-c7	MRL-DNA4	83
		BV04-01	26

* The V_H gene group refers to the reference sequences used in Fig. 1.
‡ Relevant sequences from Fig. 1 are clones that share the indicated V gene sequence.
§ The clones or hybridomas indicated (from previous publications indicated by the reference) are homologous to the indicated V region sequence.

must be a strong selection for DNA-specific B cells with antibody receptors that have arginines at these positions.

Because of its basic charge and potential ability to form hydrogen bonds with either G-C base pairs through the major groove or phosphate groups on the backbone of duplex DNA, arginine has been predicted to be important for protein binding to DNA (60). Direct evidence for such a role of arginine in anti-DNA mAb BV04-01 has been demonstrated (61). Therefore, selection for one or more arginines in VH CDR3 and at position 96 in VL CDR3 may occur because arginines at these positions increase the potential for DNA binding by the respective antibodies. Mutations that result in arginine replacements in VH CDR have been demonstrated to have marked effects on the specificity and relative affinity of anti-DNA antibodies (7, 8, 25, and Jou et al., manuscript in preparation). Moreover, the most frequent replacement mutations among anti-DNA antibodies are to arginine or asparagine (25), another amino acid predicted to be particularly impor-

Table 3. *Recurrent V_L Gene Usage among Spontaneous Anti-DNA Autoantibodies*

V_H gene group*	Relevant clones from Fig. 1‡	Hybridomas or clones from other laboratories§	Reference
$V_κ$1-MRLDNA4	17s-c2, 17s-c3, 17s-c5	MRL-DNA4, MRL-DNA10	22
	17s.93, 17s.13	DP1, 1E81	9
		BV16-19, BV04-01	26
	202.s38, 111.68	BV17-31	26
$V_κ$8-DNA5	202.9, 165.45, 165.49, 163-c1	Clone 4	7
	111-c1, 165.3, 165.5	Clone G	9
		D23	84
		Group 1	28
	202.135	A52	24
		Clone C, D20	9
$V_κ$2-BWDNA14	17.p3, 111-c2, 17s.145	BXW-DNA14	22
		B62	29
		D23	9
$V_κ$4/5-DNA22	163.72	MRL-DNA22	22
		Group 3	28
	17s-c6	BWR4	29
$V_κ$9-DNA13	163.47	DNA13	7
		33-2	25
$V_κ$10-v-16			
		DNA3,DNA4	7
		8-1	25
$V_κ$21-$V_κ$21E	17s-c4	05, 11, 12	27
$V_κ$23-DP12	165.60, 165.6	DP12	9
		Clone I	25
	165.54	D444	24
$V_κ$Ox-1-45.21.1	202.61	564, 550, 567, 563	27
	163.42	BWR5	29

* The V_L gene group refers to the reference sequences used in Fig. 2.
‡ Relevant sequences from Fig. 1 that share the indicated V gene sequence.
§ The clones or hybridomas indicated (from previous publications indicated by the reference) are homologous to the indicated V region sequence.

tant for protein binding to DNA (60). An asparagine in the binding site of mAb BV04-01 has also been demonstrated to participate in the binding of DNA by this mAb (61).

In light of these observations, the comparison of consensus VH and VL CDR sequences generated from all of the mAbs in this study generates very interesting results. To generate these results, all of the mAbs in Table 1, which would include all of the individual members of the multiple member clones (Jou et al., manuscript in preparation), were sorted into one of three categories: loDNA, ssDNA, and dsDNA (Fig. 4). The loDNA category consists of those mAbs that were not competitively inhibited from binding by ≤ 2 μg/ml of either ssDNA or dsDNA in the competitive ELISA. There are 14 IgM and two IgG mAbs in this group. The ssDNA group consists of those mAbs that were competitively inhibited by ≤ 2 μg/ml ssDNA but not dsDNA. There are 15 IgM and 31 IgG mAbs in this group. The dsDNA group consists of those mAbs, five IgM and 33 IgG, that were com-

```
                                     VH                                    |                         VL
           ------------------------------------------------------------    |  -------------------------------------------------------
           CDR1          CDR2                         CDR3                 |         CDR1             CDR2        CDR3
                                                            1          11  |
           3    3 5 5    5          56      6 9      90                00  |2  2          2 3    3 5      5 89          9
           1    5 0 2abc3           90      5 5      90abc        ijk12    |4  7abcdef8 0     4 0      6 90          7
---------------------------------------------------------------------------------------------------------------------------------
loDNA      SYGMH GI-P--GGG-TYYNDKFKG Y---G---Y   WYFDY |RSSQSLLNSRTGKNYLA WAS-RES QQSY-LPQ-T

ssDNA      SY-MN YINPGAGSGGTYYNEKFKG GGY-GGSY-Y YYFDY |RSSQSLVHSRNGNTYLH KVSNRFS QQSTHVP--T

dsDNA      SYAMS YISPKAGGGGTYYNDKFKG H-YGGRRY-   YYFDY |R-SQSLLNSRTRKNYLA WASTRAS KQSYNLP YT


dsDNA
-vh558     DYYM- YINP   -NGGTKYN-KFKG GGY-GDG    -YAFDY |-ASQSLLKSRVRSSYLH S-SNL-S HQSHRSP RT

-vh7183    SYAMS YIS-   GGGSTYYPDSVKG HYYGSRTY   --YFDY |KSSQSLLNSRTRKNYLA WASTRES KQSYNLP YT

-vhS107    DYYMN LIRNKANGYTTEYSASVKG DPYGRTRS    -TMDY |RASQ       NINIWLS KTSNLHT LQGQTYP RT

-vhQ52     SYAIS VIWT    GGDTSYNSALKS NTPLGRRY     YFDY |R-SEGAV    NIYSYLA NAKTLAE QHHYGTP PT
```

Figure 4. Consensus VH and VL CDR amino acid sequences for anti-DNA antibodies grouped according to the criteria described in the text. Determination of CDR regions is according to Kabat et al. (42). The vertically aligned numbers and letters at the top of the figure indicate amino acid positions (42). The V_H gene family representation among antibodies in the dsDNA group was 14 V_H558, 12 V_H7183, 9 V_HQ52, and 3 V_HS107. The V_L composition was 5 V_κ5; 12 V_κ8; 8 V_κ12; 3 V_κ10; 2 each V_κ9, V_λ1, and V_λ2; and 1 each V_κ1, V_κOx1, V_κ21, and V_κ19.

petitively inhibited by ≤2 μg/ml dsDNA. Of the 107 mAbs, seven were not included because either the VH or VL sequence was incomplete for each. The VH and VL CDR amino acid sequences for the mAbs in each of the three groups were aligned (42), and consensus CDR sequences for both VH and VL were generated for each group. Since there were two large clones in the dsDNA group (163-cl and 185-cl), consensus CDR sequences were also separately generated for the dsDNA mAbs according to V_H gene family expression to determine how the large clones might bias the dsDNA consensus. 163-c1 has a V_H7183 V_H, and 185-c1, a V_HQ52 V_H. As the results demonstrate (Fig. 4), the consensus VH and VL were representative of more than just the large clones.

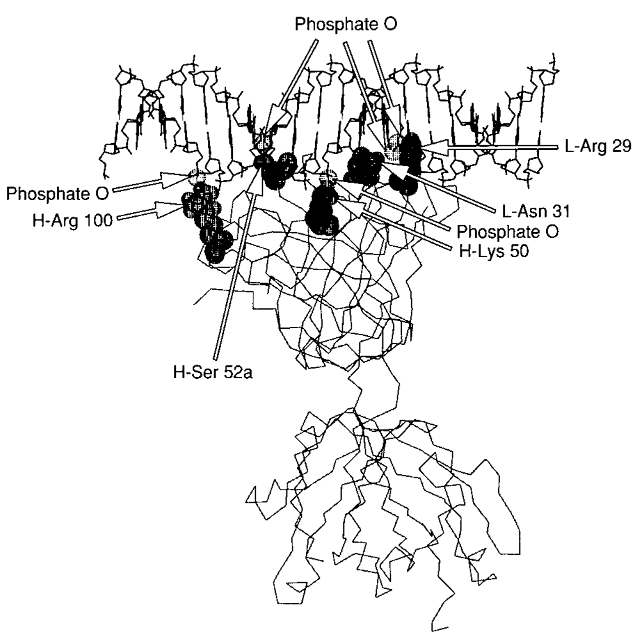

Figure 5. A hypothetical, computer-generated model of anti-DNA antibody binding to duplex, B form DNA. A model of the 163.1 mAb Fab is presented as a stick figure of the α-carbon amino acid chain without R groups. The model is based on the crystallographic coordinates for the mAb HyHEL5 (62). The DNA model is that for 16 bp of poly(dA-dT)-poly(dA-dT), B form, double-helical DNA (supplied with the software). Those amino acids in positions that would allow hydrogen bonding with the indicated phosphate oxygens on the DNA backbone are represented as dotted Van der Waals spheres of the R groups. The view presented is perpendicular to the linear axis of the DNA. Facing the model, H-Arg 100, L-Arg 29, and L-Asn 31 are on the front side of the double helix, and H-Lys 50 and H-Ser 52a are on the back side. The model was generated with the SYBYL software package (Tripos Associates, Inc., St. Louis, MO) running on a Sun 4-260 and displayed on an Evans and Sutherland PS390 graphics display terminal.

Comparison of the consensus CDR sequences for the three specificity groups of anti-DNA mAbs (Fig. 4) reveals that the loDNA group of mAbs was the most variable. The ssDNA group and dsDNA group have very similar consensus VH CDR1 and two but considerably different VH CDR3. Most interesting are the consensus arginines at positions 100 and 100a in the dsDNA group. The consensus arginines at positions 100 and 100a do not necessarily imply that most dsDNA-specific mAbs in our population have arginines at both positions. Rather, as the consensus for each V_H family demonstrates, most of the antibodies have arginine at one or the other position. Although 7 of 16 loDNA and 25 of 46 ssDNA mAbs also have arginines in VH CDR3, the arginines in VH CDR3 among these two groups are more randomly distributed. The anti-DNA mAb from DNA3, DNA4, and all the hybridomas in clone 3 previously analyzed in our laboratory (7) have arginines at one or both positions 100 and 100a in VH CDR3, and they all bind to dsDNA with high avidity. Several anti-DNA mAbs from other laboratories that bind to dsDNA also have one or more arginines at positions 100 to 100a in VH CDR3: clone F (9); clone 1 (28); and B62 and C72 (29). Although 24 of 38 IgG dsDNA-binding mAbs indicated in Table 1 have arginines in VH CDR3 between positions 99 and 100b, there are exceptions. Of the exceptions, only four have VH CDR3 without arginines.

The comparison of consensus VL CDR sequences indicates that the loDNA VL consensus is very similar to that for the dsDNA group, with the exceptions of position 29 in VL CDR1, position 55 in CDR2, and positions 89 and 93 in CDR3. The consensus VLs for both the loDNA and dsDNA groups are similar to the V_κ8 sequence of DNA5 (Fig. 2 *B*), with the differences noted above. The consensus VL for the ssDNA group is nearly identical to that of V_κ1 (Fig. 2 *A*). These consensus sequences are consistent with the binding specificities of the antibodies in the respective groups. Of 20 mAbs with a $V_\kappa8$ VL, 12 bind strongly to dsDNA, while only 1 of 26 mAbs with a $V_\kappa1$ VL binds strongly to dsDNA (Table 1, and Jou et al., manuscript in preparation). Five mAbs with $V_\kappa5$ VL also bind to dsDNA very well (Table 1, and Jou et al., manuscript in preparation). Of the five $V_\kappa5$ mAbs, four have an arginine at position 29 in VL CDR1 analogous to the $V_\kappa8$ mAbs that bind to dsDNA strongly (Fig. 2, *B* and *D*). The mAb from 202.135 and all of those from the hybridomas in clone 111-c1 have V_H558-*BWDNA7* and V_κ8-*DNA5* (Figs. 1 *A* and 2 *B*). The VH CDR3 for both clones are similar as well. The mAbs from 111-c1 all bind dsDNA (Jou et al., manuscript in preparation), whereas the mAb from 202.135 only binds to ssDNA. Given the above results, the difference in VL CDR1 position 29 between these two clones may contribute in part, if not totally, to the difference in DNA binding by these mAbs, although there are other sequence differences between these two clones. A notable position in the consensus for dsDNA-binding mAbs with VH from the V_H558 family is position 96 in VL CDR3. The consensus for this group at that position is an arginine. As detailed above, arginine at this position is probably generated by junctional diversity in the first codon of $J_\kappa1$.

The VH and VL CDR sequences for the mAbs from clone 163-c1 are nearly identical to the consensus sequences for V_H7183 dsDNA-binding antibodies (Fig. 4). Of nine mAbs from this clone, eight bind to dsDNA with relatively high avidity (Table 1, and Jou et al., manuscript in preparation). A computer model of one of the mAbs from clone 163-c1, 163.1, was generated using the crystallographic coordinates for the antilysozyme mAb HyHEL5 (62) (Fig. 5). The hypothetical binding of a model of poly(dA-dT)-poly(dA-dT) to the antibody-combining site was obtained by docking the DNA with the antibody-combining site. This DNA forms a normal B form double helix. The amino acids predicted by the computer model to be in positions to form hydrogen bonds with either base pair or backbone structures of the DNA are identified in Fig. 5. As the model illustrates, lysine at position 50, serine at position 52a, and arginine at position 100 in the heavy chain, and arginine at position 29 and asparagine at position 31 in the light chain, are each in a position that would allow them to form hydrogen bonds with phosphate oxygens on the DNA backbone. The arginines in VH position 100 and VL position 29 correspond to residues that were predicted by the dsDNA group consensus sequence (Fig. 4) to be important for dsDNA binding. Among the eight hybridomas clonally related to 163.1, a shared somatic mutation at VH position 50 and a unique mutation at VH position 52a can be directly correlated to differences in dsDNA binding by the respective mAbs (Jou et al., manuscript in preparation). Therefore, the amino acids predicted by the hypothetical model to be responsible for DNA binding by mAb 163.1 are the same ones that have been predicted to be important for dsDNA binding by structural and serological analyses of the mAbs from clone 163-c1.

The pathogenetic potential of the mAbs described in this report to initiate autoimmune disease has not been directly tested. O'Keefe et al. (63) have used the criterion of variable region cationicity, characteristic of antibodies expressing the Id564 idiotype, as an indicator of pathogenicity. Based upon this criterion, many of the mAbs described here and certainly the antibodies that bind to dsDNA with relatively high avidity would be expected to be pathogenic, as would most of the dsDNA-binding antibodies that have been reported by others, such as A52 (24), mAbs from clone A (9), and mAbs from clone H (25). This criterion is certainly consistent with the original observations of Ebling and Hahn (64) and Dang and Harbeck (65) that the anti-DNA antibodies deposited in nephritogenic mouse kidneys are cationic. The selection for basic amino acids within the V regions of most anti-DNA antibodies demonstrated here and by others (6, 7, 9, 24, 25, 29) may account for the cationicity of pathogenic anti-DNA antibodies. Of those anti-DNA mAbs that have been directly demonstrated to initiate or accelerate nephritis, H130 (66), A6.1, and 3GB3 (67) have a neutral pI. Therefore, determination of pathogenicity among anti-DNA mAbs cannot be related only to pI or idiotype expression (66, 67). Moreover, the formation of glomerular immune deposits by the mAbs H130, H241 (66), and A52 (68) were independent of DNA binding by the mAbs. These observations lead to the interesting hypothesis that the production of anti-DNA antibody is induced and sustained by DNA or DNA complexes, but pathogenesis may occur independently of DNA binding. Clearly future experiments will be needed to sort out the structural basis for the pathogenicity of anti-DNA antibody in lupus nephritis.

The most common feature among anti-DNA antibodies as demonstrated here and elsewhere (6–9, 24–27, 29, 69) is the selective expression of VH and VL structures that would be predicted and in some cases can be demonstrated to influence specificity for DNA. Although these results cannot rule out other structurally selective mechanisms such as idiotype networks and DNA crossreactive antigens that may contribute to the generation of anti-DNA, they are most consistent with the hypothesis that anti-DNA originates and is sustained by an antigen-specific immune response to DNA, most likely in a complex with proteins that could provide the necessary source for a T_H epitope.

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