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Research Article

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Structural Characteristics of the Variable Regions of Immunoglobulin Genes Encoding a Pathogenic Autoantibody in Murine Lupus

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Abstract

We have studied several monoclonal anti–double-stranded (ds) DNA antibodies for their ability to accelerate lupus nephritis in young NZB × NZW F1 female mice and to induce it in BALB/c mice. Two identified as pathogens in both strains have characteristics previously associated with nephritogenicity: expression of IgG2a isotype and IdGN2 idiotype. Both pathogenic antibodies used the combination of genes from the V_HJ558 and V_K9 subfamilies. Two weak pathogens failed to accelerate nephritis in young BW mice, but induced lupus nephritis in BALB/c mice. They both express IdGN2; one is cationic and an IgG3, the other is an IgG2a. Additional MAbs (some IgG2a, one IdGN2-positive) did not accelerate or induce nephritis. We have cloned and sequenced the variable regions of the immunoglobulin genes of one pathogenic autoantibody. No unique V, D, or J gene segments and no evidence of unusual mechanisms in generating diversity were used to construct this antibody. These data argue against use of unique abnormal Ig genes by systemic lupus erythematosus individuals to construct pathogenic autoantibody subsets. Instead, the major abnormality may be immunoregulatory. (*J. Clin. Invest.* 1990. 85:530–540.) immunoglobulin gene • pathogenic autoantibody • systemic lupus erythematosus

Introduction

In several autoimmune diseases such as systemic lupus erythematosus (SLE),[1] tissue damage is caused at least in part by autoantibodies and immune complexes containing autoantibodies (reviewed in reference 1). Therefore, there has been great interest in determining whether autoantibodies are derived from unique immunoglobulin (Ig) germline genes, and/or unique rearrangements of those genes, or, alternatively, are from somatic (hyper)mutation of antibodies originally directed against foreign antigens. To date, most data have suggested that few, if any, unique features characterize the Ig genes coding for autoantibody repertoires (reviewed in references 2 and 3). Limited sequence information has shown that both germline genes and somatically mutated genes can encode autoantibodies in SLE (4–9). In some cases, the same or similar variable region rearrangements of heavy (H) and light (L) chain can be used in antibodies binding exogenous or autologous antigens (6–8, 10–12).

Not all autoantibodies are pathogenic. Antibodies reactive with self are part of normal immune repertoires. Individuals with autoimmune diseases such as SLE have the ability to make pathogenic subsets of autoantibodies, and the inability to down-regulate them. NZB × NZW F1 female (BW) mice have been used as models of SLE nephritis which is caused at least in part by antibodies to DNA (13, 14). In studies of whole autoantibody populations in murine and human lupus, there have been conflicting data regarding the characteristics of pathogenic, nephritogenic subsets. It is generally agreed that IgG antibodies to DNA, particularly IgG isotypes that fix complement well (IgG2a, 2b, and 3 in mice), are highly associated with clinical nephritis (15–17). However, there has been disagreement regarding correlations between clinical nephritis and other features of antibodies to DNA, including epitope specificity (reactivity with single-stranded [ss]DNA, double-stranded [ds]DNA, or widely cross-reactive antigens), avidity for DNA antigens, and charge (17–26).

Several investigators have suggested that certain public idiotypes can serve as markers for pathogenic antibody subsets (23, 27–29). These opinions have been based on (*a*) identification of the idiotypes on Ig deposited in tissue lesions, including glomeruli, (*b*) delay of clinical nephritis by in vivo suppression of certain idiotypes (27, 30, 31), and (*c*) induction of lupus-like antibodies and nephritis in several normal mouse strains by in vivo up-regulation of an idiotype characteristic of human SLE (32–34). For example, we have suggested that in the BW mouse, IdGN2-positive IgG is enriched in pathogenic autoantibodies, whereas IdX-positive IgG is not. IdGN2 together with IdGN1 accounts for ~ 50% of the Ig deposited in glomeruli of nephritic BW mice; IdX for < 5% (27). IdGN2 is found more frequently than other idiotypes in the glomeruli of patients with lupus nephritis, and is infrequent in glomeruli of patients with non–lupus immune glomerulonephritis (35). Down-regulation of IdGN1 and IdGN2 is associated with delayed onset of nephritis in BW mice (27, 31). Whether the ability to make these pathogenic subsets results from genetic information unique to individuals with SLE, from immunoregulatory disorders, or from both, is unclear.

In the studies reported here, we have defined eight monoclonal antibodies (MAbs) to DNA derived from nephritic BW mice (27, 36) as either pathogens or nonpathogens, based on their ability to accelerate nephritis in young BW mice. Seven were also administered to BALB/c mice. Two MAbs were found to be pathogenic in both strains; both express IdGN2.

Cloning and sequencing of the variable regions of the H

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1. *Abbreviations used in this paper:* BUN, blood urea nitrogen; H, heavy; Id, idiotype; L, light; SLE, systemic lupus erythematosus; ss and ds, single- and double-stranded (DNA).

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and L chains of one of the pathogenic antibodies has been completed. Matches of 98% were found for both H and L variable regions with IgM antibodies to DNA that are probably not pathogens. These results suggest that a pathogenic autoantibody, like nonpathogens, does not use unique Ig germline genes, or unique variable region rearrangements of the H or L chain, or unique somatic antibody diversification.

Methods

Mice. Female NZB and male NZW mice 4–6 wk old purchased from Jackson Laboratories (Bar Harbor, ME) were raised as breeding pairs in the Rheumatology Division mouse colonies at University of California, Los Angeles. BALB/c and C57Bl/6J mice were purchased from the same supplier.

Development and testing of MAbs. Hybridomas secreting anti-dsDNA were derived by fusion of spleen lymphocytes from unimmunized nephritic BW females with either of two myeloma lines: SP2/0 or NS-1 (36, 37). Hybridomas were screened for ability to bind double-stranded calf thymus DNA in an ELISA assay. Positive MAbs were expanded in ascitic fluid of BALB/c mice after injection of pristane into peritoneal cavities. MAbs were purified from ascites after treatment with DNAase by passage through a Protein A-Sepharose column (Pharmacia, Inc., Piscataway, NJ).

Culture supernatants containing H130 were kindly provided by Drs. Kathleen Barrett, Michael Madaio, and Robert S. Schwartz, New England Medical Center, Tufts University, Boston, MA. Supernatants containing BxW-16 were kindly provided by Dr. Argyrios Theofilopoulos, Scripps Clinic, La Jolla, CA.

Isoelectric focusing. Each MAb was analyzed for pI by methods previously described (38).

ELISA assay for binding of DNA. Binding of MAbs to dsDNA was determined in an ELISA assay as described elsewhere (36). MAbs binding DNA > 3 SD above the negative control values were considered positive.

Identification of idiotypes (Ids) on MAbs. Monoclonal anti-idiotypic antibodies were developed in murine and rat systems. Anti-IdX was produced by inoculating the spleen of an LP/J mouse with an IdX-positive MAb anti-dsDNA, then fusing spleen cells with a nonsecreting murine plasmacytoma (SP2/0). Two anti-IdGN2 MAbs were used. One was made in a murine system by inoculating the spleen of an LP/J mouse with IgG anti-DNA purified from glomerular eluates of BW mice with nephritis; the second was made in a rat by direct inoculation of 100 μg of IgG of MAb A6.1 into the spleen. The rat/rat anti-IdGN2 was made by fusion of rat spleen cells with IR983F (American Tissue Culture Center, Rockville, MD). MAb anti-Ids were conjugated with alkaline phosphatase and used for assays of direct binding to MAb anti-DNA antibodies and non-DNA-binding control MAbs. The rat MAb anti-IdGN2 was used unlabeled in indirect immunofluorescence assays to detect IdGN2 deposited in glomeruli of BW mice.

The evidence for the specificity of each MAb, anti-Id, and the prevalence of each Id in BW mice, has been reported elsewhere (27, 35). Briefly, the anti-Ids do not react with allotypic, isotypic, or light chain determinants, and their interaction with target Id-positive anti-DNA antibodies can be inhibited by DNA. IdX and IdGN2 occur in BW mice and the genetically unrelated MRL/lpr and BXSB lupus strains but not in SJL, LP/J, Swiss Webster, or BALB/c strains. Approximately 60% of MAb anti-DNA derived from BW mice bear IdX or IdGN2 (or both); however, ~ 30% of IdX and 50% of IdGN2 are on non-DNA-binding Ig.

The ELISA assay for identification of these Ids on MAbs was performed as described previously (36), with MAb anti-DNA coated on polyvinyl plates followed by incubation with alkaline-phosphatase labeled anti-Ids. MAbs binding an anti-Id > 3 SD above binding by a panel of negative MAb controls were designated as positive. Some MAbs bind both anti-IdX and anti-IdGN2. Previous studies have shown that in such cases, binding of anti-IdX but not anti-IdGN2 can be blocked by preincubation of the anti-IdX with the MAb, and vice versa, indicating that these MAbs can express both idiotypes.

In vivo testing of MAbs for ability to accelerate nephritis. Purified MAbs were inoculated intraperitoneally into 18–20-wk-old female BW or C57Bl/6J mice. Each mouse had normal levels of proteinuria (< 2+ by Albustix testing) and of blood urea nitrogen (< 15 mg/dl) at the onset of this study. High levels of MAb Ig were inoculated: 100 μg twice daily, 5 of 7 d, for 3 wk for BW or 8 wk for C57Bl/6J mice. High quantities of a BW anti-DNA MAb given intermittently (100 μg once every 2 wk) can down-regulate antibodies to DNA in BW mice, probably via alteration of the idiotypic network (39). Some BW and all BALB/c (17–30 wk old) mice were inoculated i.p. with $1–2 \times 10^7$ hybridoma cells secreting MAbs of interest, rather than with purified MAb.

Mice inoculated with IgG or cells were followed at daily intervals for survival, and at 2-wk intervals for measurements of proteinuria, and blood urea nitrogen (BUN) levels. Proteinuria was measured by impregnating Albustix with urine: BUN levels were measured by impregnating Azostix with a drop of blood obtained from the retro-orbital venous plexus. Nephritis was defined throughout this study as (*a*) proteinuria > 2+ on two determinations and (*b*) BUN levels > 15 mg/dl. An additional group of 18–20-wk-old mice were inoculated with selected pathogenic or nonpathogenic MAbs (or cells) and killed at 25 wk of age. A portion of each kidney was preserved in formalin for histology, and the remainder was snap-frozen for indirect immunofluorescence studies to detect IdGN2 on Ig in glomeruli. Histologic renal damage was estimated in sections stained with hematoxylin-eosin by a blinded observer on a scale from 0 to 3+: 0 = normal glomeruli, tubules, and blood vessels; 1+ = thickening of glomerular capillary loops, small amount of round cell infiltration around vessels and tubules; 2+ = thickening of glomerular loops in all glomeruli with some smudging of structures, tubular dilatation with protein droplets in lumens, moderate round-cell infiltrate around vessels and between tubules; 3+ = obliteration of glomerular architecture in at least one glomerulus per field, marked tubular damage with dilatation, atrophy, and protein droplets within most lumens, marked round-cell infiltrates around vessels, between tubules, and around glomeruli.

Indirect immunofluorescence was performed on frozen kidney sections cut in 6-μm sections. Sections were incubated with rat MAb anti-IdGN2 (preincubated with mouse kidney powder), washed, and then incubated with fluorescein-labeled goat anti-rat IgG (Fisher Scientific Co., Pittsburgh, PA) for 45 min at room temperature. Exact conditions have been described elsewhere (35). Slides were interpreted for the presence and the intensity of fluorescence in glomeruli by a blinded observer. Individual glomeruli in each section were graded from 0 to 3+, with 0 representing no glomerular fluorescence above background and 3+ representing bright fluorescence.

To determine whether the pathogen A6.1 was deposited directly in glomeruli of BW mice, two MAbs (A6.1 and 1GE6 negative control) were radiolabeled with ^{125}I as previously described (39). Then, groups of six BW female mice (pretreated with iodine to block thyroid uptake of ^{125}I) were inoculated once with either radiolabeled A6.1 or 1GE6 as follows: group 1, inoculated at age 21 wk without other treatment; group 2, inoculated at age 22 wk after 1 wk of daily injections with 200 μg of unlabeled MAb. In previous studies (39, 40) we showed that virtually any radiolabeled MAb anti-DNA adheres to glomeruli of lupus mice 6 h after injection, but little remains at 24 h. Therefore, mice were killed 24 h after administration of the radiolabeled MAb. γ-Emissions were determined in whole liver, spleen, and kidneys from each animal. Then, glomeruli were isolated from both kidneys by methods previously described (27) and extensively washed in PBS, pH 7.2. The γ emissions from all glomeruli of each mouse were determined in a γ-counter (Beckman Instruments, Inc., Palo Alto, CA), and results were expressed as counts per minute per gram of renal tissue.

Cloning of immunoglobulin genes of hybridoma A6.1. Bacteriophage λ J1 (41) was a kind gift from Dr. Mitchell Kronenberg, University of California, Los Angeles. Phage DNA was digested with Hind III

and ligated with size-fractionated Hind III digested A6.1 DNA (9–14 kb). The ligation mixture was packaged using Gigapack Plus (Stratagene, La Jolla, CA) and 1.2 million recombinant phages were grown on the P2392 strain of bacteria. Probe preparations and hybridization conditions were as described (42, 43). The 2.0-kb Bam H-EcoRI restriction fragment containing the germline J_H3-J_H4 region was used as a J_H probe, and the 1.7-kb Hind III-Bgl II fragment containing J_K1-J_K5 germline region was used as a J_K probe. J_H- and J_K-positive clones were identified by positive signals from autoradiograms of duplicate filters.

RNA preparation and Northern blots. Total RNA from A6.1 cells was prepared (44), and polyA$^+$ RNA was selected by oligo(dT)-cellulose chromatography (45). 2 μg of polyA$^+$ RNA from A6.1 was electrophoresed in a formaldehyde gel, blotted, and probed as described (46).

Sequencing of V_H and V_K of genes of A6.1. The 1.9-kb XbaI fragment from J_H positive phage was isolated, digested with Pvu II and the resulting Xba I-Pvu II and Pvu II-Xba I fragments were subcloned into the Sma I site of the plasmid bluescript (Stratagene) in both orientations. The sequence of the V_HA6.1 region was determined from coding and noncoding strands with universal and reverse primers by dideoxynucleotide chain termination (47) using a Sequenase kit from United States Biochemicals, Cleveland, OH.

For the V_KA6.1 region, a 7-kb Xba I fragment was isolated from J_K positive phage and cloned into the Xba I site of plasmid bluescript. The sequence of V_KA6.1 region was determined using the Sequenase kit with antisense primers of 5′ C_K (corresponding to codons 116–122). This sequence was confirmed by a sense primer (5′-GCTTGGTCCCCCCTCCG) of the 5′ leader region. All primers used in this study were synthesized by Dr. Dohn Glitz of the Department of Biological Chemistry, University of California, Los Angeles.

Results

MAbs studied. Characteristics of the MAbs studied are shown in Table I. Six different IgG2a and 2b MAb anti-DNAs from nephritic BW mice were included as potential pathogens. Five additional MAbs included those from BW or other strains that were IgM, IgG1, IgG3, or non-DNA binding.

Evidence that certain MAbs are pathogenic and accelerate nephritis. Results of in vivo administration of anti-DNA-producing hybridoma B cells or purified MAbs to BW and C57Bl/6J mice are shown in Table II; results in BALB/c mice are shown in Table III. In BW mice, significant increases in the proportions of mice with clinical nephritis occurred at 25 wk of age for two MAbs, A6.1 and 3GB3. Those MAbs produced proteinuria and azotemia in 78–86% of BW mice compared with 22% of control mice at that time. Mice receiving A6.1 had significant increases in nephritis (100% vs. 76% of controls) at 30 wk of age; 3GB3 and BWds3 also produced nephritis in 100% of mice by that time; the differences were not significant. The A6.1 and 3GB3 MAbs share the following characteristics: IgG2a, expression of both IdGN2 and IdX, binding to dsDNA, and usage of gene subfamilies V_HJ558 and V_K9.

Two IdGN2+ MAbs (BWds1 and BWds3) increased the frequency of BW mice with nephritis at 25 wk (46–47% vs. 22% of controls), however, the differences were of borderline significance ($0.05 < P < 0.1$).

Several MAbs did not accelerate nephritis (1GF2, 4GH11, 5GD5, 1GE6). 1GF2, 1GE6, and 4GH11 differ from IgG2 pathogens in that they do not express IdGN2. 5GD5 expresses IdGN2 and is IgG2a. Pathogenicity had no obvious relationship to estimated binding affinity to DNA. As shown in Table I the amounts of purified antibody to achieve 50% binding of 40 ng of DNA were 10 μg for the nonpathogenic antibody 5GD5, and 10 and 100 μg for the pathogenic antibodies A6.1 and 3GB3, respectively.

As shown in Table III, the two pathogens (A6.1 and 3GB3) defined in young BW mice also induced proteinuria and azotemia in BALB/c mice. 1–4 wk after inoculation with hybridoma B cells secreting those antibodies, nephritis was present in 29–42% of mice compared to 0–9% of control mice. The most interesting comparison is between 1GE6 and A6.1. Both hybridomas grow rapidly, so that mice lived on average only 1.8 wk after implantation. However, nephritis occurred in 9% of mice receiving 1GE6 compared to 42% of mice receiving A6.1. The two MAbs (BWds1 and BWds3), which were borderline in accelerating nephritis in young BW mice, induced disease in a significant proportion of BALB/c mice. Thus, we designated BWds1 and BWds3 as weak pathogens. Three other nonpath-

Table I. Characteristics of MAbs Studied

MAb	Isotype	Idiotypes			Reactivity‖			pI	Source mouse strain	V_H	V_K
		IdGN2	IdX	Other	Mab¶	dsDNA	ssDNA				
					μg						
A6.1*	IgG2a	+	+		10	+	+	7.3	BW	J558	9
3GB3*	IgG2a	+	+		100	+	+	6.5	BW	J558	9
BWds1‡	IgG2a	+	+			+	−	7.2	BW	ND	ND
BWds3‡	IgG3	+	+		1	+	−	8.2	BW	ND	ND
4GH11	IgG2a	−	+			+	+	6.5	BW	ND	ND
1GF2	IgG2b	−	+			+	+	7.0	BW	3609	1
5GD5	IgG2a	+	+		10	+	+	7.0	BW	J558	19
1GE6	IgG1	−	−			±	±	6.3	BW	J558	1
MOPC21	IgG1	−	−			−	−	6.5	BALB/c	7183	19
H130§	IgM	−	−	H130		−	+	ND	MRL/lpr	J558	ND
BXW-16	IgM	+	+			−	+	ND	BW	J558	9

* As a result of this study, these antibodies are considered definite pathogens in that they accelerate nephritis in BW mice. ‡ As a result of this study, these MAbs are considered weak pathogens; they did not accelerate nephritis in BW mice but did induce it in BALB/c mice. § As a result of a study done by the investigators who derived this MAb, it is judged to be a nonpathogen (52). ‖ Positive and negative binding to DNA is as defined in Methods. ¶ Numbers are presented in micrograms of purified antibody to achieve 50% binding to 40 ng of dsDNA by Farr assay.

Table II. Effects of MAbs to DNA on Clinical Nephritis in BW and C57Bl/6J Mice

	Proportion with clinical nephritis		
BW mice			
Negative controls	Age: *20 wk*	*25 wk*	*30 wk*
No treatment	1/22	7/22	14/20
Normal mouse IgG	0/5	0/5	4/5
IGE6 IgG	0/5	0/5	ND
MOPC21 cells	0/9	2/9	7/8
Total (%)	1/41 (2)	9/41 (22)	25/33 (76)
Test antibodies/cells			
Definite accelerators			
A6.1 IgG	0/5	4/5	5/5
A6.1 Cells	0/17	15/17	17/17
Total (%)	0/22 (0)	19/22* (86)	22/22§ (100)
3GB3 cells	0/11	7/9‡	9/9‖ (100)
Weak accelerators			
BWds1 IgG	0/5	2/5	3/5
BWds1 cells	0/10	5/10	8/10
Total (%)	0/15 (0)	7/15¶ (46)	11/15‖ (73)
BWds3 cells	0/17	8/17¶ (47)	10/10¶ (100)
Nonaccelerators:			
1GF2 IgG	0/5	1/5	5/5
1GF2 cells	0/5	0/5	2/4
Total (%)	0/10 (0)	1/10‖ (10)	7/9‖ (78)
4GH11 cells	0/19	4/19‖ (21)	16/19‖ (84)
5GD5 cells	0/16	4/16‖ (25)	8/16‖ (50)
C57BL/6J mice			
A6.1 IgG	0/10	0/10	0/10

Antibodies to DNA or hybridoma B cells secreting those antibodies were inoculated into mice. Nephritis is defined as ≥ 2+ proteinuria (more than once) and BUN > 15 mg/dl.
* $P < 0.001$ compared to negative controls by chi square analysis; ‡ $P < 0.01$; § $P < 0.02$; ‖ $P > 0.1$, not significantly different; ¶ $0.05 < P < 0.1$.

ogens in BW mice (1GE6, 5GD5, and 4GH11) were nonpathogens in the BALB/c strain. Therefore, the analyses of definite pathogens and nonpathogens were similar in the BW mice (donors of the MAbs) and the unrelated, normal BALB/c strain. In contrast to BALB/c mice, C57Bl/6J mice did not develop nephritis after 8 wk of repeated administration of A6.1 (Table II).

In BW mice, renal damage was estimated on a scale from 0 to 3+ by examining hematoxylin-eosin-stained tissue from seven mice in the negative control group and five in the groups treated with pathogenic MAbs. Significant differences between these two groups in mean tissue damage were found; see Table IV *A*. When mice receiving A6.1 were compared to the negative controls, differences were also significant ($P < 0.05$).

Since the renal damage noted clinically and pathologically could have been due to deposition of Ig other than that inoculated, frozen kidney sections from BW mice receiving no treatment, or receiving hybridoma cells that secreted antibodies of interest were examined for deposition of IdGN2. If nephritis were caused by deposition of anti-Id, or up-regulation of an IdGN2-negative set of pathogenic idiotypes after suppression of IdGN2-positive IgG, then deposition of IdGN2-positive IgG in glomeruli should be less in the mice receiving A6.1 than in the control groups (no treatment or treated with myeloma cells). However, as shown in Table IV, the deposition of IdGN2 in glomeruli of BW mice receiving A6.1 was significantly greater than in either of the control groups by Student's *t* test, $P < 0.02$ for each comparison. No

Table III. Effects on Clinical Nephritis of Inoculation of Hybridoma B Cells into BALB/c Mice

MAb	Mice with nephritis	Weeks of survival after inoculation (mean±SEM)
	n (%)	
Controls		
No treatment	0/22 (0)	>8
1GE6	1/11 (9)	1.82±0.26
MOPC21	0/7 (0)	6.29±1.11
Pathogens		
A6.1	10/24* (42)	1.89±0.35
3GB3	5/17‡ (29)	4.24±0.64
BWds3	3/4§ (75)	2.50±0.29
BWds1	4/15‡ (27)	3.61±0.16
Nonpathogens		
5GD5	1/18 (6)	4.00±0.58
4GH11	1/5 (20)	5.20±1.16

Clinical nephritis is defined as ≥ 2+ proteinuria and BUN > 15 mg/dl.
* $P < 0.01$ compared to mice in control groups by chi square analysis; ‡ $P < 0.05$; § $P < 0.001$.

Table IV. Effect of MAbs on Renal Tissue of BW Mice

A. Histology	Score of each mouse	Mean±SEM
Negative controls		
Untreated	1, 1, 0.5	
MOPC21 cells	0, 0, 1	0.57±0.17
1GE6 IgG	0.5	
MAbs that accelerated disease		
A6.1 IgG	1, 2, 3	2.40±0.40*
3GB3 cells	3, 3	
B. Immunofluorescence	Score of each mouse	Mean±SEM
Treatment		
Untreated	1, 0.5, 1	0.83±0.17
MOPC 21 cells	0.5, 0.5, 1	0.67±0.17
A6.1 cells	2, 3, 3, 2.7, 2.5, 1.6	2.47±0.23‡
4GH11 cells	0.44, 0.57	0.5±.07

Mice were killed at 25 wk of age. (*A*) Renal damage assessed in hematoxylin-eosin stained kidney sections, on a scale from 0 to 3+. (*B*) Deposition of IdGN2 as estimated by indirect immunofluorescence of glomeruli treated with rat anti-IdGN2 followed by fluoresceinated anti-rat IgG. Scores are the average of fluorescent intensity of 30–140 glomeruli per slide estimated visually on a scale from 0 to 3+.
* $P < 0.01$ compared to negative controls by Student's *t* test;
‡ $P < 0.02$ compared to each of the two control groups.

Table V. Renal Damage of Hybridoma-bearing BALB/c Mice Assessed by Indirect Immunofluorescence of IdGN2 Expression in Glomeruli

Treatment group	Score of each mouse	Mean±SEM
I. Untreated	0, 0.17, 0.13	0.10±0.05
II. MOPC21	0, 0.2	0.10±0.10
III. 1GE6	0.06, 0.1	0.08±0.02
IV. A6.1	1.29, 1.0, 1.07, 0.8	1.04±0.10*

* $P < 0.01$ by comparing group IV to each of the other groups by rank sum analysis (the Mann-Whitney U test).

significant difference was found comparing the amount of IdGN2 in glomeruli in BW mice receiving 4GH11 to that in either control group.

Glomeruli of BALB/c mice were assessed by indirect immunofluorescence for the expression of IdGN2. We previously demonstrated that IdGN2 is not expressed in sera of BALB/c mice (27). As shown in Table V, A6.1 hybridoma-bearing BALB/c mice had significantly higher ($P < 0.001$) IdGN2-positive Ig deposited in glomeruli than did BALB/c mice either untreated or inoculated with myeloma MOPC21 or hybridoma 1GE6. The simplest explanation for the presence of IdGN2 in glomeruli of BALB/c mice is the direct deposition of IdGN2-positive MAbs secreted by the injected hybridoma cells.

Deposition of injected antibody in glomeruli. The increased expression of IdGN2 observed in glomeruli of BW mice inoculated with A6.1 cells could be due to (*a*) direct deposition of pathogenic MAb or (*b*) enhanced production of IdGN2 positive Ig of the host. To distinguish between these two possibilities, we followed the tissue distribution of injected ^{125}I-labeled MAb. As shown in Table VI, repeated inoculations of the pathogenic MAb, A6.1, resulted in deposition of large quantities in glomeruli. In contrast, repeated inoculations of the nonpathogenic MAb 1GE6 did not. The differences were seen both before and after 1 wk of repeated inoculations. Before injections A6.1 was found in significantly higher quantities than 1GE6 in both kidneys and liver, suggesting it might be a "sticky antibody." However, after 1 wk of inoculations A6.1 was present in whole kidneys in more than five times the quantities of 1GE6, and in glomeruli about threefold higher. However, quantities of the two MAbs deposited in liver were similar. These data suggest that the pathogenic MAb was deposited directly in glomeruli.

We conclude from these data that A6.1 is a pathogenic autoantibody which can accelerate nephritis in BW mice and induce it in BALB/c mice, probably by direct deposition in glomeruli.

Cloning of pathogenic autoantibody A6.1. We obtained a genomic clone of A6.1 containing the variable regions of Ig genes. Southern analyses were performed on genomic DNA from BW liver, parental myeloma Sp2/0, and A6.1 hybridoma cells to detect restriction fragments containing the rearranged V-(D)-J genes by hybridization to J_H or J_K probes. The expressed A6.1 V_H and V_K genes were contained on unique 10- and 12-kb Hind III fragments, respectively (data not shown). The expressed fragments were different in size from those derived from the germline liver sample and the rearranged myeloma SP2/0. Thus we isolated 9–14-kb Hind III digested genomic A6.1 DNA to make a λ bacteriophage library.

J_H and J_K positive clones were mapped with restriction sites; results are shown in Fig. 1. A 1.9-kb Xba I fragment as depicted in Fig. 1 *A* was isolated from the J_H-positive phage to probe a Northern blot containing A6.1 RNA. Another strip with the same amount of A6.1 RNA was probed with cDNA containing the IgG2b constant region which hybridized to both of the highly homologous IgG2a and IgG2b messages (48). A common band ~ 2.1 kb was found hybridizing to both probes as shown in Fig. 2 *A*. We have previously cloned a nonproductively rearranged V_HDJ_H gene from A6.1 cells which did not hybridize to the polyA$^+$ RNA from A6.1 in this experiment (data not shown). Thus, the 1.9-kb Xba I fragment contains the V_HDJ_H gene expressed by A6.1. Similarly,

Table VI. Increased Deposition of the Pathogenic MAb A6.1 in Glomeruli of BW Mice Compared to the Nonpathogenic MAb 1GE6

	Mean portion total radioactivity found in tissues at 24 h (±SEM)*					
	Whole kidneys		Glomeruli		Liver	
	A6.1	1GE6	A6.1	1GE6	A6.1	1GE6
	%					
Group 1						
Before repeated MAb injections	1.38±0.45*	0.41±0.03	1.09	0.26	0.32±0.05*	0.12±0.03
Ratio	3.37:1		4.19:1		2.66:1	
Group 2						
After 1 wk of daily MAb injections	1.42±0.09‡	0.50±0.03	5.32	1.91	0.43±0.08	0.52±0.30
Ratio	5.32:1		2.80:1		0.829:1	

* Data are recorded as mean percent of total injected radioactivity (MAb A6.1 or 1GE6 radiolabeled with ^{125}I) found in 0.3 g of tissue 24 h after inoculation. This was calculated as: (cpm/0.3 g kidney or liver)/(cpm inoculated into animal) × 100. Each group contained six mice. Data listed represent mean percent of total counts per minute for each of 12 kidneys, and each of six livers in each group. Glomeruli were isolated from all kidneys in each group, pooled, and reported as a single value. ‡ Differences between the two MAb-treated groups were statistically different by Student's *t* test, $P < 0.05$ or less.

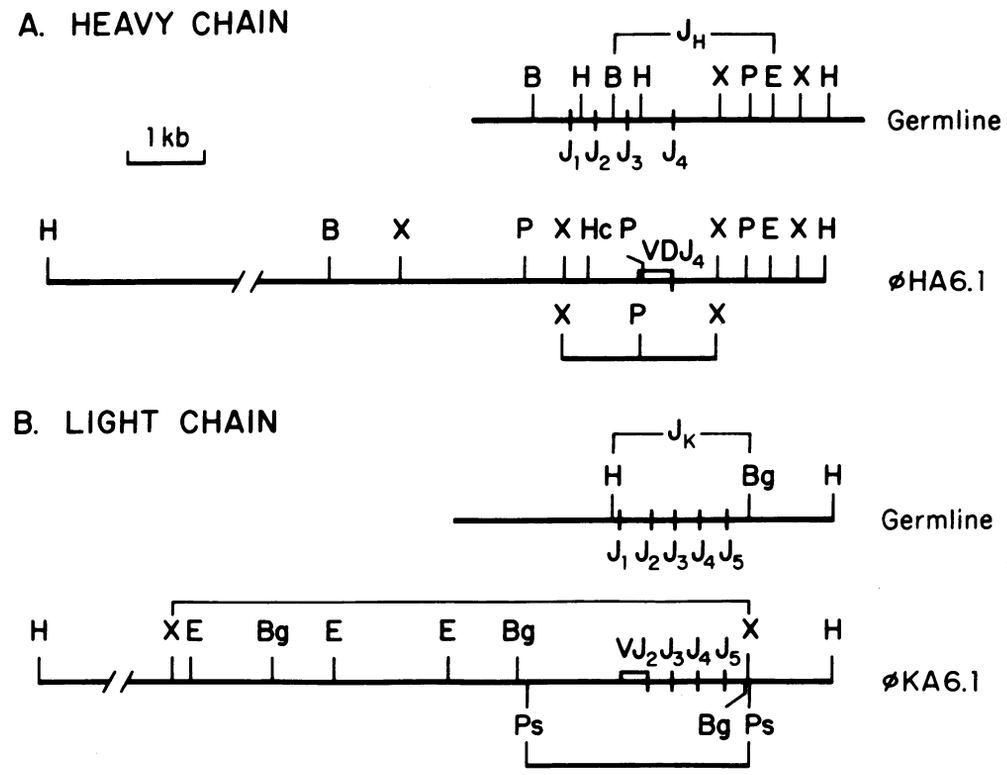

Figure 1. Comparison of restriction enzyme maps of the germline J genes to the cloned genomic DNA fragments of bacteriophages containing the expressed V-(D)-J immunoglobulin genes from A6.1 cells. The probes are (*A*) the marked J_H region for the heavy chain and (*B*) the bracketed region of J_K for the light chain. Restriction enzyme sites are: E, Eco RI; B, Bam H; Bg, Bg III; H, Hind III; Hc, Hinc II; P, Pvu II; Ps, Pst I; X, Xba I.

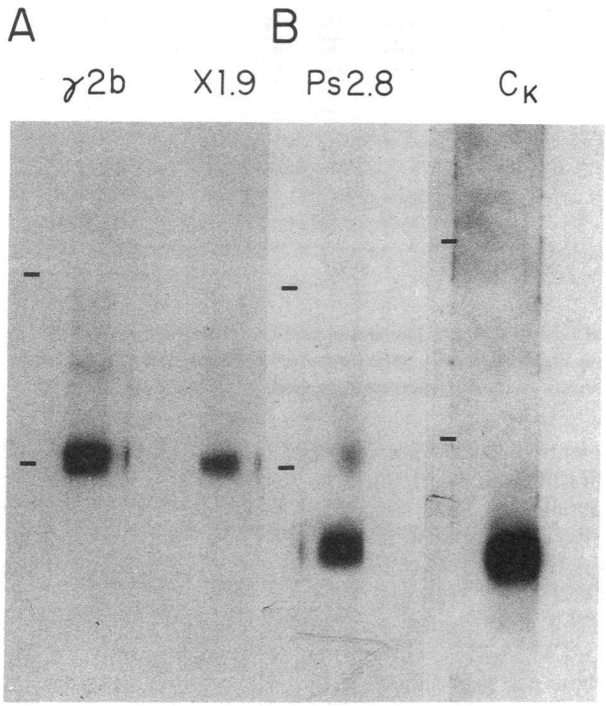

Figure 2. Northern blot of RNA from A6.1 cells. Each lane contained 2 μg of polyA$^+$ RNA from A6.1 cells and was electrophoresed in a denaturing agarose gel. The gel was blotted onto a nitrocellulose filter and each lane was cut into strips. In *A* one strip was hybridized with [^{32}P]IgG2b cDNA (73) and the other with the 1.9-kb Xba I fragment from phage HA6.1 as depicted in Fig. 1 *A*. In *B* one strip was probed with the 2.8-kb Pst I fragment from phage KA6.1 as indicated in Fig. 1 *B* and the other with a labeled 2.8-kb Bgl II fragment containing the genomic constant region of κ chain (74). The bars indicate the position of ribosomal RNA of the size of 2 and 5 kb.

A6.1 RNA strips were hybridized to a C_K probe and a 2.8-kb Pst I fragment as shown in Fig. 1 *B* from J_K-positive phage separately. An RNA species of about 1.2 kb, the expected size of Igκ messages, was detected by each probe (Fig. 2 *B*). Thus, the 2.8-kb fragment contains the expressed V_KJ_K of A6.1.

Sequencing of the V_H region of A6.1. The 1.9-kb XbaI fragment of the expressed V_H gene was subcloned and the sequence of the A6.1 V_H gene was determined as shown in Fig. 3. The A6.1 V_H was a member of the largest V_H subfamily, J558 (49), and was 98% homologous to the expressed V_H of H130 (5). The monoclonal H130 (IgM, κ) binds ssDNA (50) and bears a dominant public Id of MRL/lpr mice (51), a murine SLE model genetically unrelated to BW mice. H130 has been shown in another system to be nonnephritogenic (52). A6.1 and H130 had differences in six nucleotides which resulted in four differences in amino acids scattered in the V_H framework regions. The V_H H130 probably uses a germline gene in that its sequence is identical to the sequenced portion (amino acids 4–92) of a BALB/c germline J558 V_H gene, H18 (53). The sequences of D and J_H4 segments of A6.1 are homologous to the corresponding BALB/c germline sequences (54, 55). At the V_H-D_H junction the sequence GATTCCCC, and at the D-J_H junction sequence GGG could be generated by N region addition (56). The D region of H130 is derived from the SP2 family; H130 used the same J_H4 segment as A6.1. Thus, owing to usage of different D regions and N region addition, the CDR3 regions of the two antibodies are very different. In general, in Ig genes the V-D-J recombination event retains the 5′ flanking V_H region, and the different restriction maps of the 5′ flanking region of these two V_H genes (Fig. 1 and reference 5) imply that these two V_H derived from closely related but different germline genes. The V_K region of H130 is not known.

Since IdGN2 and IdH130 are found concentrated in the kidney eluates in BW and MRL/lpr mice, respectively, we

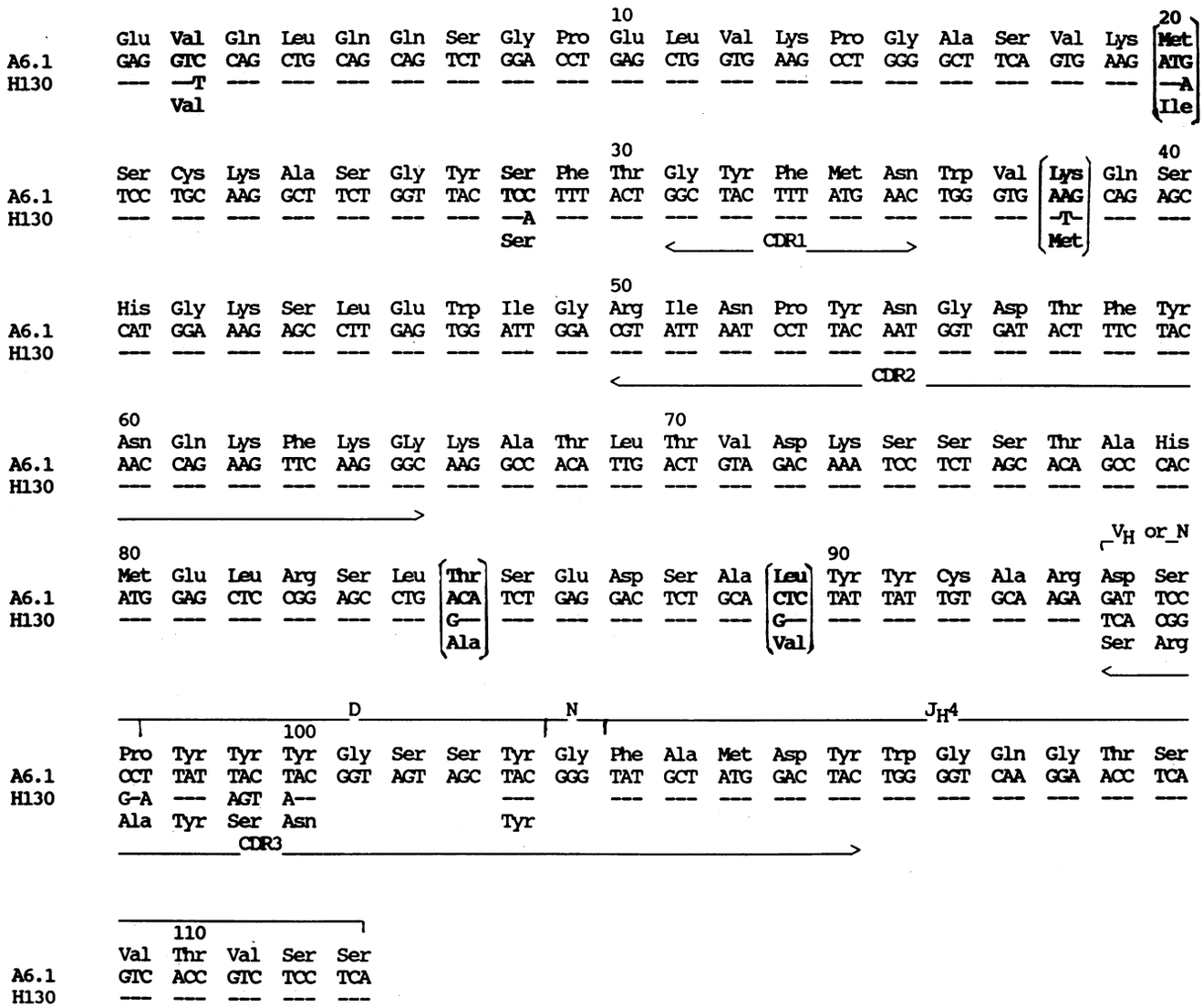

Figure 3. Sequence of expressed A6.1 V_H genes and its comparison to expressed H130 V_H genes. The amino acid translation appears on the top of the sequence, numbering according to that of Kabat et al. (75). Identities with the expressed V_H gene from A6.1 cells are indicated as dashes, vertical brackets mark the codon containing unmatched nucleotide(s). CDR, complementarity determining region.

compared the cross-reactivity of these two Ids. Anti-IdH130 did not bind A6.1 IgG; anti-IdGN2 did not bind H130 IgM in ELISA assays (data not shown). Therefore the idiotypes on H130 and A6.1 are different, which could be due to differences in V_K or D_H.

Sequencing of the V_K region of A6.1. A 7-kb Xba I fragment containing the expressed V_K region was subcloned into plasmids, and V_K and J_K segments were sequenced. The V_K gene of A6.1 was derived from the V_K9 family. Sequence comparison revealed a 98% match between V_K genes of A6.1 and BXW-16 (11) as shown in Fig. 4. They may have derived from the same germline V_K9 gene. Hybridoma BXW-16 (IgM, κ) secretes antibodies to ssDNA and is derived from a 6-mo-old BW mouse (11). It has not been tested for pathogenicity in any system; since it is an IgM it is unlikely that it can cause nephritis. Both antibodies used the J_K2 gene, thus the sequences of V_K-J_K regions of A6.1 and BXW-16 were also 98% identical. Interestingly, the V_H of BXW-16 is also derived from a J558 subfamily with a 91% match to the V_H of A6.1. The D-J elements of BXW-16 are DFL16.1 (same as A6.1) and J_H2 (different from A6.1). Since the variable regions of these two antibodies are quite similar, we asked whether they express similar idiotypes. Both anti-IdGN2 and anti-IdX reacted strongly with BXW-16 in ELISA assays (data not shown) thus BXW-16 is IdGN2+ and IdX+.

The most similar germline V_K9 gene published is the MOPC41 V_K (57), with only an 86% match to A6.1 V_K. The 41-base pair (bp) differences are scattered in both framework and hypervariable regions. The ratios of replacement to silent mutations in frameworks (12/3) and in complementarity determining regions (12/2) are both high, suggesting that the V_K of A6.1 is probably derived from a different germline member of V_K9.

Discussion

The major purpose of these studies was to determine whether any unique genetic information is used to assemble pathogenic

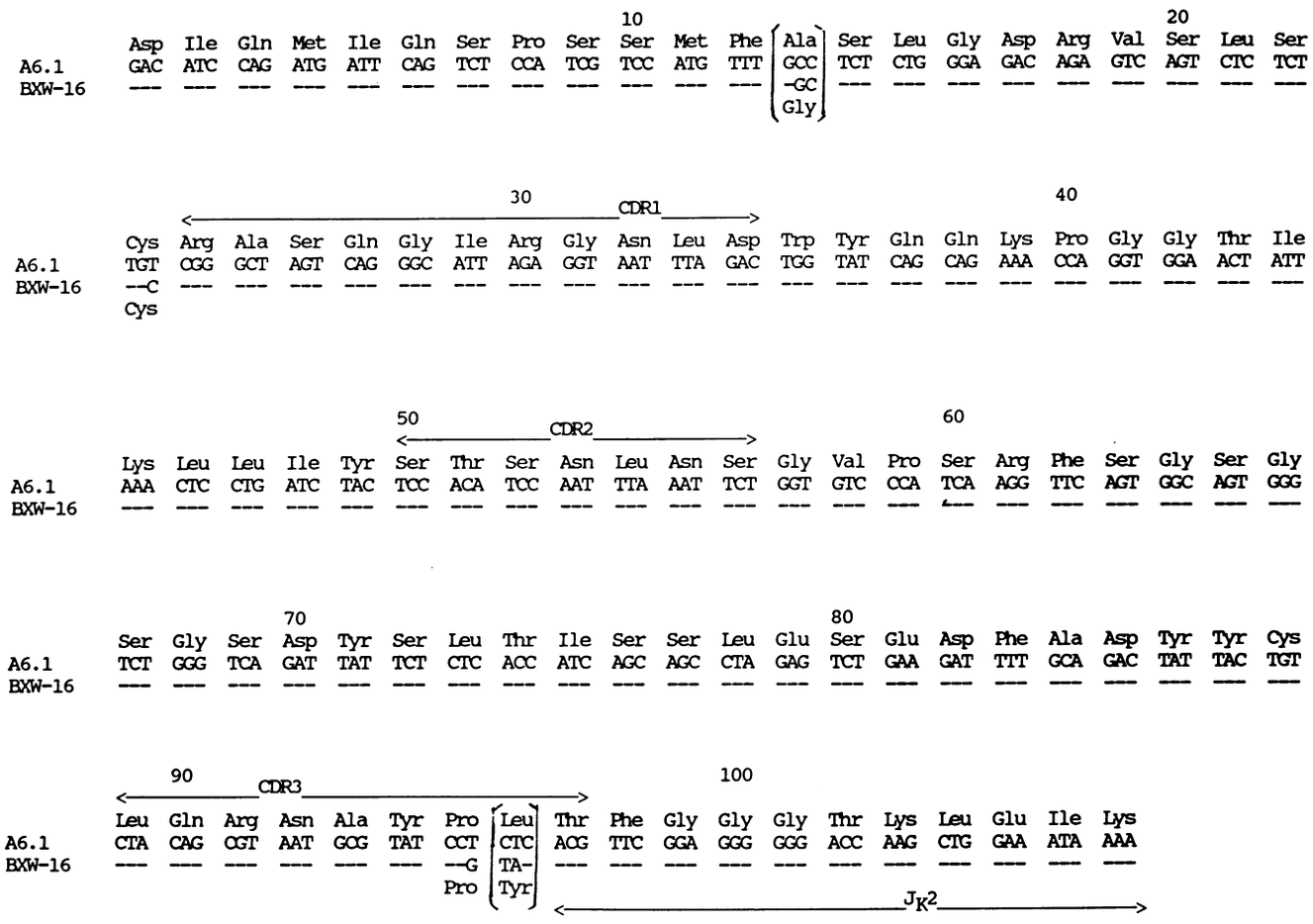

Figure 4. Sequence of expressed A6.1 V_K genes and its comparison to expressed BXW-16 V_K genes. Symbols as described in the legend of Fig. 3.

autoantibodies. To clearly distinguish between pathogenic and nonpathogenic autoantibodies, we established an in vivo assay system in which high blood levels of different antibodies to DNA were sustained in young BW, C57Bl/6J, or BALB/c mice. Two of the anti-DNA MAbs tested significantly accelerated the onset of nephritis in BW and induced it in BALB/c mice. One of these definite pathogens, A6.1, was chosen for cloning and sequencing of the variable regions of heavy and light chains. The two pathogens have characteristics that have been associated with nephritogenicity; they are IgG2a, express IdGN2 (as well as IdX), and bind dsDNA with relatively high avidity. They are not cationic. Two weak pathogens are found to induce lupus nephritis in normal BALB/c mice, but failed to accelerate disease in BW mice. The difference in pathogenicity observed in these two strains of mice may be due to the high background spontaneous nephritis of BW mice, masking the effect of weak accelerators. One of the weak pathogens is cationic (pI 8.2) IgG3; it expresses both IdGN2 and IdX, and binds dsDNA. The other weak pathogen is IgG2a, expresses IdGN2 and IdX. Two other MAbs, 5GD5 and 4GH11, are clearly nonpathogenic in both strains tested. Puzzlingly, 5GD5 has characteristics of pathogens: it is IgG2a, expresses IdGN2 and IdX, and binds dsDNA. Thus, multiple factors may determine the ability of an antibody to cause nephritis. Isotype, charge, polyreactivity, binding avidity, and complement-fixing properties may each play a role. Studies are in progress to compare polyreactivity and complement-fixing ability of 5GD5 to A6.1. Sequencing of each of these MAbs may provide important information regarding regions in H and/or L chains that are associated with pathogenicity.

It should be noted that A6.1 was a pathogen only in BW and BALB/c mice. Prolonged administration of purified MAb A6.1 to C57Bl/6J mice did not induce proteinuria or azotemia. Similarly, the BALB/c strain, but not the C57BL/6 strain tested by Mendolovic et al. (32–34) developed SLE when inoculated with the human MAb 16/6 lupus idiotype. In their system the susceptibility of the murine strains is controlled by genes other than those linked to either major histocompatibility complex (MHC) or heavy chain allotypes and may depend on the ability of different strains to make anti-Ids (33, 34). However, in another model of SLE, mice undergoing chronic graft-vs.-host reactions make large quantities of IgG2a antibodies to DNA, but the ability of those antibodies to induce nephritis is strongly influenced by the recipient's MHC class II genes (58, 59). Until more mouse strains are tested with our pathogenic autoantibodies, we do not know whether MHC-linked genes are involved in susceptibility. The pathogenicity of four different MAbs observed in normal BALB/c mice argues against the possibility that BW mice express an unique antigen in their kidneys. It is likely that the ability to produce a

nephritogenic autoantibody subset is both necessary and sufficient to induce lupus nephritis in a susceptible genetic background.

The Ig genes encoding anti-DNA antibodies derived from mice predisposed to lupus, and from patients with the disease, have been studied by many investigators. Most genetic studies were carried out in lupus-prone mice, and no disease association was found with any particular IgH- or Igκ-V haplotype (2, 3, 11). Furthermore, no unique features of Ig gene repertoire have been detected: (*a*) restriction fragment length polymorphism studies of Ig heavy chain and kappa light chain loci of autoimmune mice reveal that both loci are inherited unaltered from their nonautoimmune ancestors (11, 60, 61), (*b*) analyses of variable regions of Ig genes encoding antibodies to DNA demonstrate the same variable region families that are present in normal mice and are used to encode antibodies to foreign antigens (6, 10–12, 36, 62–64). In agreement with these observations, we found no unique V, D, or J gene segments used in a pathogenic autoantibody. The sequence of V_HA6.1 is 98% identical to the nonpathogenic V_HH130, and is > 90% identical to V_H used by normal antibodies to alpha 1 → 6 dextran (65). It is possible that pathogenic antibodies are derived from a unique germline subfamily of V_HJ558. Interestingly, in lupus-prone BW and MRL/lpr mice, preferential usage of V_HJ558 is observed as mice age (36, 66), which could result from overexpression of all V_HJ558 subfamilies or of selected subsets. Thus far we have ruled out two J558 subfamilies, *p*-azophenylarsenate A (67) and H4a-3 (68, 69), which are mapped proximally to D-J regions and have < 85% sequence homology to V_HA6.1.

Until the germline counterpart of V_HA6.1 is cloned, we cannot directly address the role of somatic mutation in generating pathogenic autoantibody. Behar and Scharff (7) have cloned both NZB and NZW germline S107-V_H11 genes from which five BW anti-DNA MAbs derived their V_H genes. Comparing R/S ratios in complementarity determining regions and frameworks they concluded the extent of somatic mutations is similar to those observed in BALB/c antibodies elicited by influenza virus which use the germline S107-V_H11 gene (7). The nucleotide sequences of the S107-V_H11 gene from NZB and NZW mice are identical, 8 of 290 nucleotides in the mature coding region are polymorphic in the BALB/c germline gene. The six-nucleotide difference of V_HA6.1 and BALB/c germline V_HH18 gene may be partially due to polymorphism; the high degree of sequence match makes it less likely that somatic hypermutation is the mechanism of generating this pathogenic autoantibody.

The V_KA6.1 sequence is most similar to V_K BXW-16 (98%), the next best match is to the V_K region of an anti-lysozyme antibody (88%) (70). It is interesting to note the best-matched V_H and V_K genes to V genes of A6.1 are both found in antibodies to DNA derived from lupus mice, suggesting that common mechanisms cause the expansion of these B cell clones.

Comparison of V_H sequence homology between A6.1 and 8 other published antibodies to DNA derived from lupus mice using V_HJ558 shows considerable heterogeneity (78–98% match), similar to that observed in antibodies to various antigens (5, 8, 10–12) (four nearly identical V_H sequences encoding clonally related MAbs are considered as one from reference 8). At least five D genes and all four J_H segments are used to encode murine antibodies to DNA with various numbers (5–16) of amino acids in the CDR3 region. Besides BXW-16, none of the other 13 anti-DNA antibodies (8, 11, 12) sequenced use the V_K9 gene. Thus, there is considerable structural diversity in generating binding sites for DNA.

There are similarities among anti-DNA MAbs. The predicted amino acid sequences of the CDR3 region of A6.1 contain a motif of YYGSS; remarkably similar motifs are also found in three murine (U4, BXW-7, BXW-16) and three human (18/2, 21/28, TH3, Kim4.6) anti-DNA antibodies (6, 11, 71, 72). This motif may be important in IdGN2 expression, since it is present in IdGN2+ A6.1 and BXW-16 but absent in IdGN2-H130. IdGN2 is a conformational idiotype, its expression relies on both chains of Ig. In our library of anti-DNA MAbs 10/11 IdGN2 expressing hybridomas use V_HJ558 with four V_K subfamilies (30, and Table I). We are in the process of sequencing the expressed V regions of the other IdGN2+ MAbs attempting to define the structural basis of the idiotope.

In one computer-modeled three-dimensional anti-ssDNA antibody structure, the antigen-combining site is surrounded by all complementarity determining regions (CDR) except L2, resulting in lining both the walls and base of the cleft with a preponderance of arginine and tyrosine residues (12). The D segment of A6.1 derived from the germline DFL-16 is using a reading frame which encodes four tyrosines. In addition, there is the fifth tyrosine in CDR3 encoded by J_H4. There are three basic residues in CDR2, 1 and 5 tyrosines in CDR1 and CDR3 of the heavy chain; and a total of three arginine residues in CDR1 and three of the light chains of A6.1. These residues may participate in electrostatic and hydrogen bonding interactions with the sugar phosphate backbone of DNA.

The data in this paper show that no unique genetic components—i.e., V, D, or J gene segments—are used to comprise pathogenic autoantibodies since one can find evidence that the same elements are used in nonpathogenic and even normal antibodies. However, the data here and elsewhere are consistent with the possibility that particular combinations of common elements may lead to a pathogenic autoantibody. The occurrence of V_HJ558 with a particular D and V_K9 in pathogenic BW autoantibodies may be an example of such a "bad" combination. Similarly, the normal processes of N region addition and/or somatic mutation may generate pathogenic autoantibodies. Since current evidence suggests that formation of different combinations, N regions and somatic mutations appears to be random or nearly so, this argues that the defect in autoimmune mice is their inability to down-regulate the expression of these pathogenic combinations, which are generated by normal mechanisms.

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References

1. Theofilopoulos, A. N., and F. J. Dixon. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37:269–390.

2. Bona, C. A. 1988. V genes encoding autoantibodies: molecular and phenotypic characteristics. *Annu. Rev. Immunol.* 6:327–358.

3. Kofler, R., F. J. Dixon, and A. N. Theofilopoulos. 1987. The genetic origin of autoantibodies. *Immunol. Today.* 80:374–380.

4. Dersimonian, H., R. S. Schwartz, K. J. Barrett, and B. D. Stollar. 1987. Relationship of human variable region heavy chain germ-line genes to genes encoding anti-DNA autoantibodies. *J. Immunol.* 139:2496–2501.

5. Trepicchio, W., Jr., A. Maruya, and K. J. Barrett. 1987. The heavy chain genes of a lupus anti-DNA autoantibody are encoded in the germ line of a nonautoimmune strain of mouse and conserved in strains of mice polymorphic for this gene locus. *J. Immunol.* 139:3139–3145.

6. Giusti, A. M., N. C. Chien, D. J. Zack, S.-U. Shin, and M. D. Scharff. 1987. Somatic diversification of S107 from an antiphosphocholine to an anti-DNA autoantibody is due to a single base change in its heavy chain variable region. *Proc. Natl. Acad. Sci. USA.* 84:2926–2930.

7. Behar, S. M., and M. D. Scharff. 1988. Somatic diversification of the S107 (T15) V_H11 germ-line gene that encodes the heavy-chain variable region of antibodies to double-stranded DNA in (NZB × NZW)F_1 mice. *Proc. Natl. Acad. Sci. USA.* 85:3970–3974.

8. Shlomchik, M. J., A. H. Aucoin, D. S. Pisetsky, and M. G. Weigert. 1987. Structure and function of anti–DNA autoantibodies derived from a single autoimmune mouse. *Proc. Natl. Acad. Sci. USA.* 84:9150–9154.

9. Chen, P. P., M. Liu, S. Sinha, and D. A. Carson. 1988. A 16/6 idiotype-positive anti-DNA antibody is encoded by a conserved V_H gene with no somatic mutation. *Arthritis Rheum.* 31:1429–1431.

10. Kofler, R., D. J. Noonan, D. E. Levy, M. C. Wilson, N. P. H. Moller, F. J. Dixon, and A. N. Theofilopoulos. 1985. Genetic elements used for a murine lupus anti–DNA autoantibody are closely related to those for antibodies to exogenous antigens. *J. Exp. Med.* 161:805–815.

11. Kofler, R., R. Strohal, R. S. Balderas, M. E. Johnson, D. J. Noonan, M. A. Ducholsal, F. J. Dixon, and A. N. Theofilopoulos. 1988. Immunoglobulin K light chain variable region gene complex organization and immunoglobulin genes encoding anti-DNA autoantibodies in lupus mice. *J. Clin. Invest.* 82:852–860.

12. Eilat, D., D. M. Webster, and A. R. Rees. 1988. V region sequences of anti–DNA and anti–RNA autoantibodies from NZB/NZW F1 mice. *J. Immunol.* 141:1745–1753.

13. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of glomerulonephritis of NZB/W mice. *J. Exp. Med.* 127:507–522.

14. Koffler, D., P. H. Schur, and H. G. Kunkel. 1967. Immunological studies concerning the nephritis of Systemic lupus erythematosus. *J. Exp. Med.* 126:607–624.

15. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. N. Izui, C. B. Wilson, P. S. McConahue, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine lupus-like syndromes. *J. Exp. Med.* 148:1198–1215.

16. Rothfield, N. F., and B. D. Stollar. 1967. The relation of immunoglobulin class, pattern of antinuclear antibody, and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus. *J. Clin. Invest.* 46:1785–1794.

17. Ebling, F. M., and B. H. Hahn. 1980. Restricted subpopulations of DNA antibodies in kidneys of mice with systemic lupus: comparison of antibodies in serum and renal eluates. *Arthritis Rheum.* 23:392–403.

18. Yoshida, H., M. Yoshida, S. Izui, and P. H. Lambert. 1985. Distinct clonotypes of anti–DNA antibodies in mice with lupus nephritis. *J. Clin. Invest.* 76:685–694.

19. Jacob, L., M.-A. Lety, R. C. Monteiro, F. Jacob, J.-F. Bach, and D. Louvard. 1987. Altered cell-surface protein(s), crossreactive with DNA, on spleen cells of autoimmune lupus mice. *Proc. Natl. Acad. Sci. USA.* 84:1361–1363.

20. Winfield, J. B., I. Faiferman, and D. Koffler. Avidity of anti–DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus. Association of high avidity antinative DNA antibody with glomerulonephritis. *J. Clin. Invest.* 59:90–96.

21. Steward, M. W., F. E. Katz, and N. J. Wost. 1975. The role of low affinity antibody in immune complex disease: The quantity of anti-DNA antibodies in NZB/W F1 hybrid mice. *Clin. Exp. Immunol.* 21:121–130.

22. Dang, H., and R. J. Harbeck. 1984. The in vivo and in vitro glomerular deposition of isolated anti–double-stranded-DNA antibodies in NZB/W mice. *Clin. Immunol. Immunopathol.* 31:265–278.

23. Galvalchin, J., R. A. Seder, and S. K. Datta. 1987. The NZB × SWR model of lupus nephritis. I. Cross-reactive idiotypes of monoclonal anti-DNA antibodies in relation to antigenic specificity, charge and allotype: identification of inter-connected idiotype families inherited from the normal SWR and the autoimmune NZB parents. *J. Immunol.* 138:128–137.

24. Pankewycz, O. G., P. Migliorini, and M. P. Madaio. 1987. Polyreactive autoantibodies are nephritogenic in murine lupus nephritis. *J. Immunol.* 139:3287–3294.

25. Sabbaga, J., S. R. P. Line, P. Potocnjak, and M. P. Madaio. 1989. A murine nephritogenic monoclonal anti-DNA autoantibody binds directly to mouse laminin, the major non-collagenous protein component of the glomerular basement membrane. *Eur. J. Immunol.* 19:137–143.

26. Raz, E., M. Brezis, E. Rosenmann, and D. Eilat. 1989. Anti–DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. *J. Immunol.* 142:3076–3082.

27. Hahn, B. H., and F. M. Ebling. 1987. Idiotypic restriction in murine lupus: high frequency of three public idiotypes on serum IgG in nephritic NZB/NZW F1 mice. *J. Immunol.* 138:2110–2118.

28. Shoenfeld, Y., and D. Isenberg. 1987. DNA antibody idiotypes: a review of their genetic, clinical and immunopathologic features. *Sem. Arthritis Rheum.* 16:245–252.

29. Solomon, G., J. Schiffenbauer, H. D. Keiser, and B. Diamond. 1983. Use of monoclonal antibodies to identify shared idiotypes on human antibodies to DNA from patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* 80:850–854.

30. Hahn, B. H., and F. M. Ebling. 1984. Suppression of murine lupus nephritis by administration of an anti-idiotypic antibody to anti–DNA. *J. Immunol.* 132:187–190.

31. Ebling, F. M., D. G. Ando, N. Panosian-Sahakian, K. C. Kalunian, and B. H. Hahn. 1988. Idiotypic spreading promotes the production of pathogenic autoantibodies. *J. Autoimmun.* 1:47–61.

32. Mendlovic, S., S. Brocke, Y. Shoenfeld, M. Ben-Bassat, A. Meshorer, R. Bakimer, and E. Mozes. 1988. Induction of a systemic lupus erythematosus-like disease in mice by a common human anti–DNA idiotype. *Proc. Natl. Acad. Sci. USA.* 85:2260–64.

33. Mendlovic, S., H. Fricke, Y. Shoenfeld, and E. Mozes. 1989. The role of anti–idiotypic antibodies in the induction of experimental systemic lupus erythematosus in mice. *Eur. J. Immunol.* 19:729–734.

34. Mendlovic, S., S. Brocke, Y. Shoefeld, R. Bakimer, and E. Mozes. 1989. The genetic regulation of the induction of experimental SLE in mice. *J. Immunol.* In press.

35. Kalunian, K. C., N. Panosian-Sahakian, F. M. Ebling, A. H. Cohen, J. S. Louie, J. Kaine, and B. H. Hahn. 1989. Idiotypic characteristics of immunoglobulins associated with human systemic lupus erythematosus: studies of antibodies deposited in glomeruli. *Arthritis Rheum.* 32:513–522.

36. Panosian-Sahakian, N., J. L. Klotz, F. M. Ebling, M. Kronenberg, and B. H. Hahn. 1989. Diversity of immunoglobulin variable gene segments found in pathogenic and non-pathogenic anti-DNA

autoantibodies from a single (NZB × NZW) F1 mouse. *J. Immunol.* 142:4500–4506.

37. Hahn, B. H., F. M. Ebling, S. Freeman, B. Clevinger, and J. Davie. 1980. Production of monoclonal antibodies to DNA by somatic cell hybrids. *Arthritis Rheum.* 23:942–945.

38. Hahn, B. H., and F. M. Ebling. 1984. A public idiotypic determinant is present on spontaneous cationic IgG antibodies to DNA from mice of unrelated lupus-prone strains. *J. Immunol.* 133:3015–3019.

39. Hahn, B. H., and F. M. Ebling. 1983. Suppression of NZB/NZW murine nephritis by administration of a syngeneic monoclonal antibody to DNA: possible role of anti-idiotypic antibodies. *J. Clin. Invest.* 71:1728–1736.

40. Hahn, B. H. 1982. Characteristics of pathogenic subpopulations of antibodies to DNA. *Arthritis Rheum.* 25:747–752.

41. Mullins, J. I., D. S. Brody, R. C. Binari, and S. M. Cotter. 1984. Viral transduction of c-*myc* gene in naturally occurring feline leukemias. *Nature (Lond.).* 308:856–858.

42. Wahl, G. M., M. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. USA.* 76:3683–3687.

43. Hodgson, C. P., and R. Z. Fisk. 1987. Hybridization probe size control: optimized "oligolabelling." *Nucleic Acids. Res.* 15:6295.

44. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

45. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 197–199.

46. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201–5205.

47. Sanger, F., S. Nicklen, and A. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

48. Ollo, R., C. Auffray, C. Morchamps, and F. Rougeon. 1981. Comparison of mouse immunoglobulin gamma 2a and gamma 2b chain genes suggests that exons can be exchanged between genes in a multigenic family. *Proc. Natl. Acad. Sci. USA.* 78:2442–2446.

49. Livant, D., C. Blatt, and L. Hood. 1986. One heavy chain variable region gene segment subfamily in the BALB/c mouse contains 500–1000 or more members. *Cell.* 47:461–470.

50. Andrzejewski, C., Jr., J. Rauch, E. Lafer, B. D. Stollar, and R. S. Schwartz. 1981. Antigen-binding diversity and idiotypic cross-reactions among hybridoma autoantibodies to DNA. *J. Immunol.* 126:226–231.

51. Rauch, J., E. Murphy, J. B. Roths, B. D. Stollar, and R. S. Schwartz. 1982. A high frequency idiotypic marker of anti-DNA autoantibodies in MRL-lpr/lpr mice. *J. Immunol.* 129:236–241.

52. Madaio, M. P., J. Carlson, J. Cataldo, A. Ucci, P. Migliorini, and O. Pankewycz. 1987. Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. *J. Immunol.* 138:2883–2889.

53. Schiff, C., M. Milili, and M. Fougereau. 1985. Functional and pseudogenes are similarly organized and may equally contribute to the extensive antibody diversity of the Ig V_HII family. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:1225–1230.

54. Kurosawa, Y., and S. Tonegawa. 1982. Organization, structure, and assembly of immunoglobulin heavy chain diversity DNA segments. *J. Exp. Med.* 155:201–218.

55. Sakano, H., R. Maki, Y. Kurosawa, W. Roeder, and S. Tonegawa. 1980. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. *Nature (Lond.).* 286:676–683.

56. Alt, F. W., and D. Baltimore. 1982. Gene segments: Joining of immunoglobulin heavy chains: implications from a chromosome with evidence of three D-Jy fusions. *Proc. Natl. Acad. Sci. USA.* 79:4118–4122.

57. Seidman, J. G., E. E. Max, and P. Leder. 1979. A k-immunoglobulin gene is formed by site-specific recombination without further somatic mutation. *Nature (Lond.).* 280:370–375.

58. Gleichmann, H., E. G. Gleichmann, J. Andre-Schwartz, and R. S. Schwartz. 1972. Chronic allogeneic disease III. Genetic requirements for the induction of glomerulonephritis. *J. Exp. Med.* 135:516–532.

59. Portanova, J. P., F. M. Ebling, W. S. Hammond, B. H. Hahn, and B. L. Kotzin. 1988. Allogeneic MHC antigen requirements for lupus-like autoantibody production and nephritis in murine graft-vs-host disease. *J. Immunol.* 141:3370–3376.

60. Kofler, R., R. M. Perlmutter, D. J. Noonan, F. J. Dixon, and A. N. Theofilopoulos. 1985. Ig heavy chain variable region gene complex of lupus mice exhibits normal restriction fragment length polymorphism. *J. Exp. Med.* 162:346–351.

61. Trepicchio, W., Jr., and K. J. Barrett. 1985. The IgH-V locus of MRL mice: restriction fragment length polymorphism in eleven strains of mice as determined with V_H and D gene probes. *J. Immunol.* 134:2734–2739.

62. Trepicchio, W., Jr., and K. J. Barrett. 1987. Eleven MRL-lpr/lpr anti-DNA autoantibodies are encoded by genes from four V_H gene families: a potentially biased usage of V_H genes. *J. Immunol.* 138:2323–2331.

63. Kofler, R. 1988. A new murine Ig V_H gene family. *J. Immunol.* 140:4031–4034.

64. Klotz, J. L., G. L. Kiser, and M. Kronenberg. 1988. Molecular and serological diversity of anti-DNA autoantibodies from NZB and (NZB × NZW) F1 mice. *Immunol. Lett.* 19:341–350.

65. Akolkar, P. N., S. K. Sikder, S. B. Bhattacharya, J. Liao, F. Gruezo, S. L. Morrison, and E. A. Kabat. 1987. Different V_L and V_K germ-line are used to produce similar combining sites with specificity for alpha (1-> 6) dextrans. *J. Immunol.* 138:4472–4479.

66. Foster, M. H., M. MacDonald, K. J. Barrett, and M. P. Madaio. 1989. Preferential Usage of J558 V_H genes for autoantibody production in murine lupus. *FASEB J.* 3:A301. (Abstr.).

67. Rathbun, G. A., F. Otani, E. C. B. Milner, J. D. Capra, and P. W. Tucker. 1988. Molecular characterization of the A/J J558 family of heavy chain variable region gene segments. *J. Mol. Biol.* 202:383–395.

68. Brodeur, P. H., G. E. Osman, J. J. Mackle, and T. M. Lalor. 1988. The organization of the mouse Igh-V locus: dispersion, interspersion, and the evolution of V_H gene family clusters. *J. Exp. Med.* 168:2261–2278.

69. Schiff, C., M. Milli, I. Hue, S. Rudikoff, and M. Fougereau. 1986. Genetic basis for expression of the idiotypic network: one unique Ig V_H germline accounts for the major family of Ab1 and Ab3 (Ab1') antibodies of the GAT system. *J. Exp. Med.* 163:573–587.

70. Darsley, M. J., and A. R. Rees. 1985. Nucleotide sequences of five anti-lysozyme monoclonal antibodies. *EMBO (Eur. Mol. Biol. Organ) J.* 4:393–398.

71. Dersimonian, H., K. P. W. J. McAdam, C. Mackworth-Young, and B. D. Stollar. 1989. The recurrent expression of variable region segments in human IgM anti-DNA autoantibodies. *J. Immunol.* 142:4027–4033.

72. Cairns, E., P. C. Kwong, V. Misener, P. Ip, D. A. Bell, and K. A. Siminovitch. 1989. Analysis of variable region genes encoding a human anti-DNA antibody of normal origin: implications for the molecular basis of human autoimmune responses. *J. Immunol.* 143:685–691.

73. Bothwell, A. L. M., M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, and D. Baltimore. 1981. Heavy chain variable region contribution to the NP^b family of antibodies: Somatic mutation evident in a gamma2a variable region. *Cell.* 24:625–637.

74. Max, E. E., J. V. Maizel, Jr., and P. Leder. 1981. The nucleotide sequence of a 5.5-kilobase DNA segment containing the mouse k immunoglobulin J and C region genes. *J. Biol. Chem.* 256:5116–5120.

75. Kadat, E., T. Wu, M. Reid-Miller, H. Perry, and K. Gottesman. 1987. Sequences of Proteins of Immunological Interest. 4th edition. U. S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD. 508–525.