

# Light Chain Usage in Anti-double-stranded DNA B Cell Subsets: Role in Cell Fate Determination

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## Summary

Two major mechanisms for the regulation of autoreactive B cells that arise in the bone marrow are functional silencing (anergy) and deletion. Studies to date suggest that low avidity interactions between B cells and autoantigen lead to B cell silencing, whereas high avidity interactions lead to deletion. Anti-double stranded (ds) DNA antibodies represent a pathogenic autoantibody specificity in Systemic Lupus Erythematosus (SLE). An understanding of their regulation is critical to an understanding of SLE. We now demonstrate in a transgenic model in which mice express the heavy chain of a potentially pathogenic anti-DNA antibody that antibody affinity for dsDNA does not alone determine the fate of anti-dsDNA B cells. B cells making antibodies with similar affinities for dsDNA are regulated differently, depending on light chain usage. A major implication of this observation is that dsDNA may not be the self antigen responsible for cell fate determinations of anti-dsDNA B cells. Light chain usage may determine antigenic cross-reactivity, and cross-reactive antigens may regulate B cells that also bind dsDNA.

Anti-double stranded (ds)<sup>1</sup> DNA antibodies are characteristic of the autoimmune disease SLE and titers of IgG anti-dsDNA antibodies in patients' serum correlate with disease activity and nephritis. Analyses of the immunoglobulin variable region gene loci reveal no differences between autoimmune and nonautoimmune mouse strains and no differences in human kindreds that associate with autoimmune disease. Furthermore, the immunoglobulin variable region (V) genes used in both murine and human anti-DNA antibodies are also used in the generation of a protective antibody repertoire (1-5).

Studies of the regulation of autoreactive B cells became possible with the advent of transgene technology. Analyses of B cells expressing transgene encoded autoantibodies have demonstrated the existence of several mechanisms for maintaining self tolerance: functional silencing or anergy, deletion, and receptor editing (6-13). Based on investigations from several laboratories, Goodnow has proposed that there are thresholds of receptor occupancy that correlate with different mechanisms of regulation (14). According to this model, deletion occurs under conditions of extensive receptor cross-linking, whereas silencing occurs under conditions of more moderate cross-linking.

To study the regulation of anti-dsDNA antibodies, we previously generated nonautoimmune BALB/c and NZW

mice transgenic for the  $\gamma 2b$  heavy chain of the R4A anti-dsDNA antibody. The R4A antibody is encoded by an S107 V11 heavy chain gene and a Vk1 light chain gene, binds dsDNA, and deposits in glomeruli of SCID mice (15, 16). In R4A- $\gamma 2b$  transgenic BALB/c and NZW mice, negligible anti-DNA activity is present in the serum, and fusion of unstimulated splenocytes from these mice fails to yield transgene expressing anti-dsDNA hybridomas. Anti-dsDNA B cells, however, are present in the spleens of these mice and can be activated *in vitro* by LPS to secrete transgene encoded anti-dsDNA antibody. Furthermore, R4A anti-dsDNA hybridomas can be obtained from these mice if splenocytes are stimulated *in vitro* with LPS before fusion (9, 17).

In the present study we compared transgene expression in nonautoimmune BALB/c and NZW mice and autoimmune NZB/W F1 mice. While negligible transgene-encoded anti-DNA activity is present in the serum of BALB/c and NZW mice, such activity is present in the serum of all NZB/W F1 mice. Analyses of hybridomas show that transgene expressing anti-dsDNA B cells from NZB/W F1 mice use a broad spectrum of light chain genes. In contrast, anti-dsDNA B cells from nonautoimmune mice use almost exclusively Vk1 genes. Thus, two populations of anti-dsDNA B cells exist, which are differentially regulated in nonautoimmune mice. There is a Vk1 anti-dsDNA subset that is present but is functionally silent, and a non-Vk1 subset which is targeted for deletion. In the NZB/W F1 autoimmune background, both populations are activated *in vivo*. Since the Vk1 and the non-Vk1 anti-dsDNA anti-

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<sup>1</sup>Abbreviations used in this paper: ds, double-stranded; FR1, framework 1; ss, single-stranded; V, variable region.

bodies have similar affinities for dsDNA, this critical, potentially pathogenic, specificity cannot be regulated solely by binding to dsDNA. Alternative models of regulation in which cell fate is determined by light chain usage need to be considered.

## Materials and Methods

**Transgenic Mice.** Mice expressing the R4A- $\gamma$ 2b heavy chain transgene have been previously reported (9, 17). Transgene expressing NZB/W F1 mice were generated by breeding transgenic NZW mice with wild-type NZB mice.

**Generation of Hybridomas.** Splenocytes derived from two 8-wk-old unimmunized transgenic NZW mice and two unimmunized transgenic BALB/c mice were fused after stimulation for 48 h in vitro with LPS (17).

Six fusions were performed using spleen cells from eight NZB/W F1 transgenic mice ranging in age from 2.5–10 mo. Three fusions were performed with naive spleen cells; two were performed with LPS stimulated cells. In one fusion, half of the splenocytes were stimulated with LPS for 48 h before fusion and the other half were fused without prior exposure to LPS. Hybridomas were screened by ELISA for  $\gamma$ 2b dsDNA binding as previously described (17). Cells from hybridoma wells displaying anti-dsDNA activity were cloned in soft agar.

**Analysis of V Gene Expression.** Hybridoma clones were screened for expression of R4A- $\gamma$ 2b and Vk1 genes by RNA dot blot using probes specific for the mouse S107 and Vk1 gene families as previously described (17). A Vk1 probe was provided by Dr. C. Schildkraut (Albert Einstein College of Medicine, Bronx, New York; reference 18).

Total RNA was isolated from hybridomas (Ultraspec RNA kit; Biotecx Labs., Houston, TX). First strand cDNA for the R4A heavy chain and  $\kappa$  light chain genes were synthesized using 10  $\mu$ g of RNA and 100 ng of antisense oligonucleotide primers specific for the mouse gamma constant region (TGGACAGGGA/CTC-CAG/TAGTTC) and for the mouse  $\kappa$  light chain constant region (ACACTCATTCTCTGTTGAA). PCR amplification was performed using vent polymerase. For the R4A heavy chain gene, an oligomer specific for framework 1 (FR1; GGTGAAGCTG-GTGGAACTCTGG) and the gamma constant region primer were used; for kappa light chain genes a Vk1, FR1-specific oligomer (CCAGCAGTGATGTTGTGATGACCC) or a cocktail of degenerate FR1, Vk oligomers (19), and the  $\kappa$  constant region primer were used. PCR products were sequenced with the dsDNA cycle sequencing system (GIBCO BRL, Gaithersburg, MD) using constant region, FR1, and internal primers. Sequencing reactions were electrophoresed on 6 or 7% acrylamide gels.

**Allelic Exclusion.** Allelic exclusion of anti-dsDNA hybridomas was determined by ELISA as previously described (17). In brief, 96-well Falcon plates (Becton Dickinson, Lincoln Park, NJ) were coated with a 1/1,000 dilution of goat anti-mouse antibody to IgM, IgG1, IgG3, IgG2a, or IgG2b (Southern Biotechnology Assoc., Birmingham, AL). After blocking plates in PBS, 1.0% BSA, cell supernatants normalized to 10  $\mu$ g/ml were added to wells for 1 h at 37°C followed by the addition of goat anti-mouse IgM, IgG1, IgG3, IgG2a, or IgG2b antibody, respectively, conjugated to alkaline phosphatase (Southern Biotechnology Assoc.). All ELISA washes were performed with PBS-0.05% Tween. ELISAs were developed with *p*-nitrophenylphosphate disodium as substrate (Sigma Chemical Co., St. Louis, MO) and ELISAs were read at 405 nm in a Titertek-Multiscan ELISA reader.

**Antibody Quantitation.** IgG2b antibodies in hybridoma supernatants were quantitated by ELISA as previously described (16).

**Analysis of Antigenic Specificity.** Quantitated hybridoma supernatants were tested by ELISA for binding to dsDNA and single stranded (ss) DNA. The dsDNA ELISA was performed on salmon sperm DNA-coated Immulon 2 ELISA plates (Dynatech Labs. Inc., Chantilly, VA) according to Iliev et al. (17). The ss-DNA ELISA was performed as described for dsDNA (17) except that salmon sperm DNA was not filtered through a nitrocellulose filter and was denatured by boiling and quick cooled on ice before coating Immulon 2 plates.

**Affinity Measurements of Anti-dsDNA Antibodies.** Affinity constants for selected anti-dsDNA antibodies were determined according to an antigen inhibition ELISA as described by Nieto et al. (20). Immulon 2 plates were coated with 100  $\mu$ g/ml salmon sperm DNA and blocked in PBS, 1.0% BSA. Hybridoma supernatants diluted to an antibody concentration that was within the linear range on an anti-dsDNA antibody titration curve, were then incubated in wells of ELISA plates along with increasing concentrations of soluble salmon sperm DNA used as inhibitor (0.0–2 mg/ml) for 2 h at 37°C. Wells were washed and incubated for 1 h at 37°C with goat anti-mouse IgG2b conjugated to alkaline phosphatase and developed with substrate solution as described above. ELISA measurements were read at 405 nm in a Titertek Multiscan ELISA reader. Apparent affinities were calculated based on the concentration of soluble DNA resulting in 50% inhibition of antibody binding (20).

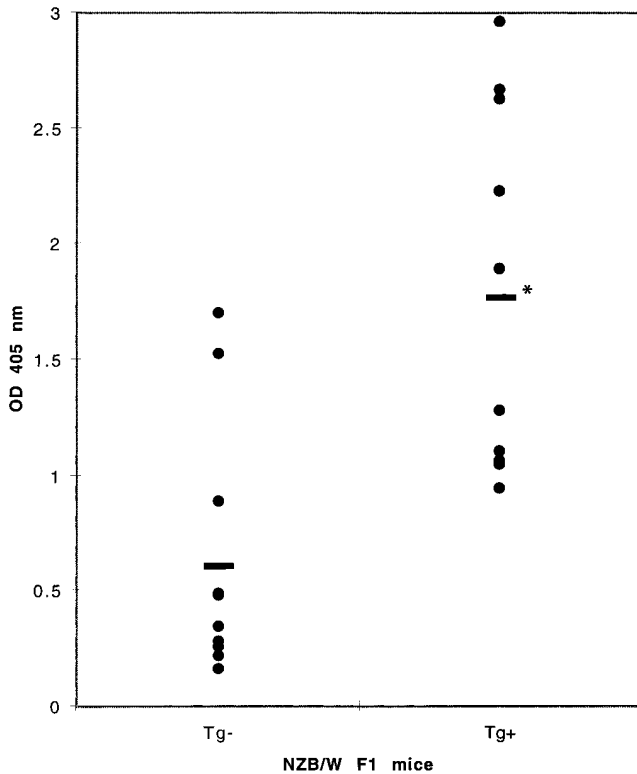
Dissociation constants were determined according to the method of Friguet et al. (21). In brief, linearized plasmid DNA was nick translated with  $^{32}$ P-labeled deoxynucleotide triphosphates. A constant amount of antibody was combined with various amounts of radiolabeled DNA (5–100 ng), brought to a final volume of 1.0 ml with SSC, and then incubated for 3 h at room temperature. Antibody-dsDNA complexes were trapped on 0.45  $\mu$ m type HA millipore filters (Millipore Corp., Bedford, MA). Bound radioactivity was detected on a liquid scintillation counter. Results were plotted and dissociation constants were obtained from the slope of the linear regression.

**Pathogenicity of Anti-dsDNA Antibodies.** Hybridoma cells ( $5 \times 10^6$ ) from two lines, BW7E6H1 and NZW14E10, were injected intraperitoneally into pristane-primed SCID mice. 3 wk after injections, animals were killed, and kidneys removed and imbedded in paraffin. Sections were stained with biotin-conjugated goat anti-mouse IgG (Vectastain ABC kit; Vector Labs., Inc., Burlingame, CA) and then developed with NBT-BCIP substrate (GIBCO BRL) according to Katz et al. (16). Stained sections were viewed with a Zeiss microscope.

## Results

**Generation of Hybridomas from Nonautoimmune Mice.** While splenocytes from nonautoimmune NZW and BALB/c R4A- $\gamma$ 2b transgenic mice can be induced to secrete transgene encoded anti-dsDNA antibodies by in vitro stimulation with LPS, spontaneous secretion of  $\gamma$ 2b anti-dsDNA antibody is negligible in these mice (9). In contrast, NZB/W F1 R4A- $\gamma$ 2b transgenic mice have elevated titers of  $\gamma$ 2b anti-dsDNA. These antibodies are transgene encoded; at 2.5–3 mo of age, elevated titers of  $\gamma$ 2b DNA binding activity is present only in the sera of transgenic NZB/W F1 mice and not in nontransgenic littermates (Fig. 1).

To understand the differential regulation of anti-dsDNA



**Figure 1.** Measurement of R4A- $\gamma$ 2b anti-dsDNA antibody in the sera of NZB/W F1 transgenic and nontransgenic mice. Sera from 10 transgenic ( $Tg^+$ ) NZB/W F1 mice and 10 nontransgenic ( $Tg^-$ ) littermates were diluted 1/500 and then tested by ELISA for binding of  $\gamma$ 2b antibody to dsDNA. Ages of  $Tg^+$  and  $Tg^-$  mice range from 10–21 wk. There is no linear relationship between age of the mouse and anti-dsDNA titer within these populations. \*Mean value significantly exceeds mean value for  $Tg^-$  mice.  $P < 0.002$  (Student's  $t$  test).

B cells in autoimmune and nonautoimmune mice, we generated transgene encoded anti-dsDNA hybridomas. We were able to obtain anti-dsDNA producing hybridomas from NZW or BALB/c mice only if splenocytes were cultured with LPS before fusion (17). 5–10% of hybridomas from LPS-stimulated splenocytes expressing the R4A- $\gamma$ 2b transgene bound dsDNA. 20 anti-dsDNA hybridomas, 9 from NZW mice, and 11 from BALB/c mice, were analyzed in detail. Expression of the transgene was verified in these clones by sequencing through the VDJ junction of the  $\gamma$ 2b heavy chain in all hybridomas and throughout the entire V region in half. Sequence analysis revealed the heavy chain to be encoded by the transgene in all cases and further revealed that there was no somatic mutation of the R4A heavy chain.

To examine the endogenous light chains expressed in these hybridomas, we first looked for expression of a Vk1 transcript by RNA dot blot since the “wild-type” R4A antibody possesses a Vk1 light chain (15). 17 of 20 lines expressed a Vk1 light chain. All light chains were sequenced (Fig. 2) confirming the light chain identification obtained by RNA dot blot. The Vk1-A gene was used in the majority (12) of hybridomas. 16 of the Vk1 light chain genes were somatically mutated. The three non-Vk1 anti-dsDNA associated light chains, BA12BB2, BA12BD-1, and BA1227,

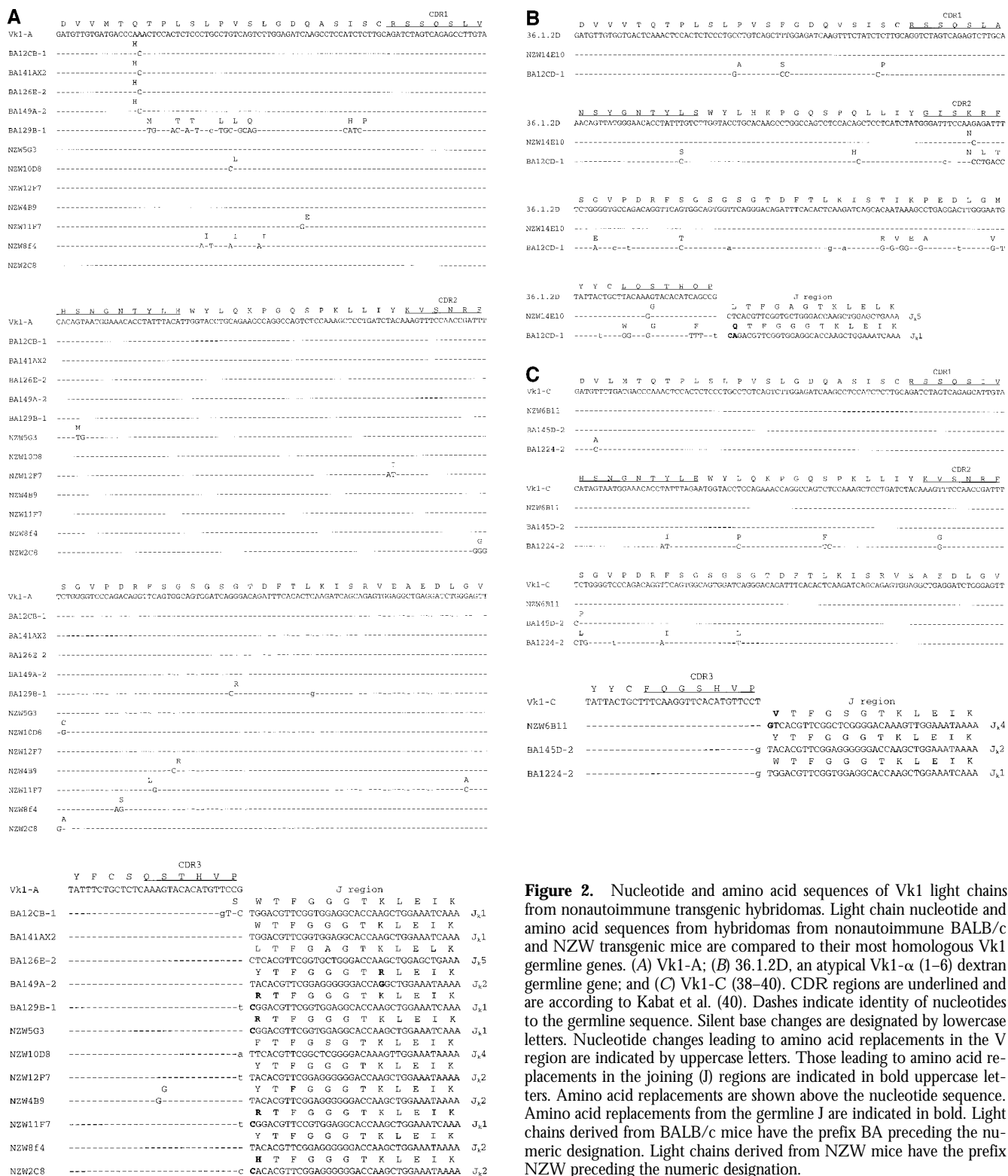
were derived from BALB/c hybridomas and are encoded by Vk21, Vk2, and Vk4/5 genes respectively (data not shown).

**Generation of Hybridomas from NZB/W F1 Autoimmune Mice.** Transgene expressing anti-dsDNA hybridomas from R4A- $\gamma$ 2b transgenic NZB/W F1 mice could be obtained from non-LPS activated B cells as well as from LPS activated splenocytes, confirming activation in vivo of anti-dsDNA B cells in the autoimmune mice. In contrast to the dominant Vk1 usage seen in nonautoimmune mice, only 5 of 16 autoimmune derived anti-dsDNA hybridomas use a Vk1 gene (Table 1). 12 cell lines were obtained from LPS-stimulated splenocytes, of which only 4 (33%) use Vk1 genes. Only 1 of 4 cell lines obtained from unstimulated NZB/W F1 splenocytes (25%) uses a Vk1 light chain. Therefore, there is little difference in the frequency of Vk1 expression in hybridomas from LPS-stimulated and unstimulated splenocytes. Vk1 genes from NZB/W F1 hybridomas display no somatic mutation in three (BW19G10, BW21D11, and BW10D9) of the five Vk1 genes sequenced (Fig. 3). The base change observed at the junction of Vk and Jk in Bw19G10 and BW21D11 is likely the result of imprecise joining during Vk-Jk rearrangement.

The remaining 11 hybridomas use light chains from eight different non-Vk1 light chain gene families (Fig. 4), including two from the Vk10 family and three from the Vk4/5 family. Since none of the non-Vk1 genes share 100% homology with any germline genes reported in the EMBL/GenBank/DDBJ database, we cannot determine whether they are somatically mutated or whether they represent novel germline genes.

We previously observed that R4A anti-dsDNA hybridomas from nonautoimmune mice display a lack of allelic exclusion, whereas the transgene expressing non-dsDNA binding hybridomas all display intact allelic exclusion (17). We, therefore, examined the R4A anti-dsDNA hybridomas from NZB/W F1 mice for expression of endogenous  $\mu$ ,  $\gamma$ 1,  $\gamma$ 3, or  $\gamma$ 2a heavy chain. Hybridoma supernatants were assayed by ELISA for binding to anti-isotype antibodies. Most hybridomas (14 of 16) were observed to express endogenous heavy chain as well as the R4A- $\gamma$ 2b heavy chain. In nine hybridomas, the endogenous heavy chain is of the  $\mu$  isotype; in 5, it is of the  $\gamma$ 3 or  $\gamma$ 2a isotype. All hybridomas failing to display allelic exclusion demonstrated expression of a second isotype as detected by an OD reading that was at least fivefold above background levels ( $OD_{405\text{ nm}} > 0.6$ ). Two hybridomas from NZB/W F1 mice, BW10D9 and BW7E6, demonstrated no binding above background to anti- $\mu$ ,  $\gamma$ 1,  $\gamma$ 3, or  $\gamma$ 2a ( $OD_{405\text{ nm}} < 0.150$ ) suggesting that they maintained allelic exclusion or that they secreted an endogenous  $\gamma$ 2b heavy chain.

**Comparison of Vk1 and Non-Vk1 Anti-DNA Antibodies.** Comparison of the light chain repertoire of transgene-encoded anti-dsDNA antibodies used by the nonautoimmune and autoimmune mice (Table 1) demonstrates a statistically significant dominance of Vk1 light chains in hybridomas from the nonautoimmune BALB/c and NZW mice. To understand how R4A-Vk1 anti-DNA antibodies might differ from R4A non-Vk1 anti-DNA antibodies, we compared



**Figure 2.** Nucleotide and amino acid sequences of Vk1 light chains from nonautoimmune transgenic hybridomas. Light chain nucleotide and amino acid sequences from hybridomas from nonautoimmune BALB/c and NZW transgenic mice are compared to their most homologous Vk1 germline genes. (A) Vk1-A; (B) 36.1.2D, an atypical Vk1- $\alpha$  (1-6) dextran germline gene; and (C) Vk1-C (38-40). CDR regions are underlined and are according to Kabat et al. (40). Dashes indicate identity of nucleotides to the germline sequence. Silent base changes are designated by lowercase letters. Nucleotide changes leading to amino acid replacements in the V region are indicated by uppercase letters. Those leading to amino acid replacements in the joining (J) regions are indicated in bold uppercase letters. Amino acid replacements are shown above the nucleotide sequence. Amino acid replacements from the germline J are indicated in bold. Light chains derived from BALB/c mice have the prefix BA preceding the numeric designation. Light chains derived from NZW mice have the prefix NZW preceding the numeric designation.

binding to dsDNA by ELISA (Fig. 5). A similar spectrum of binding was present among antibodies with both Vk1 and non-Vk1 light chains. Several non-Vk1 antibodies displayed lower dsDNA binding than Vk1 antibodies, demonstrating that the non-Vk1 antibodies did not represent a

higher affinity subset. We also measured the apparent affinity of some representative Vk1 and non-Vk1 anti-dsDNA antibodies in a DNA inhibition assay (20). There was no difference in the range of affinities among the Vk1 and the non-Vk1 anti-dsDNA antibodies (Table 2). One antibody

**Table 1.** *Vk Gene Usage*

Strains	Total No.	Vk1 (%)	Non-Vk1 (%)
BALB/c	11	8 (73)	3 (27)
NZW	9	9 (100)	0 (0)
NZB/W F1	16	5 (31)*	11 (69)
LPS stimulated	12	4 (33)	8 (66)
Unstimulated	4	1 (25)	3 (75)

\*Significantly less than NZW ( $P < 0.002$ ) and BALB/c ( $P < 0.05$ ) Pearson  $\chi^2$  test.

which binds only moderately well to dsDNA-coated plates (BW16B2) by direct ELISA, appears to have a higher apparent affinity, as detected by a DNA inhibition ELISA, than expected. This antibody may bind better to DNA in solution than to DNA in a solid phase, a characteristic of some anti-DNA antibodies which has previously been observed (22).

Because it has been reported that B cells with specificity for ssDNA are tolerized by anergy induction, while B cells binding dsDNA are deleted (8), we assayed for cross-reactivity with ssDNA by ELISA (Table 3). At 2  $\mu\text{g/ml}$ , one Vk1 anti-dsDNA antibody, BA129B-1, derived from a BALB/c mouse, and two non-Vk1 anti-dsDNA antibodies, BW14C7B1 and BW9B5F7, derived from an NZB/W F1 mouse bind strongly to ssDNA (Table 3) relative to R4A. Two other anti-dsDNA antibodies, one from a BALB/c mouse and one from an NZB/W F1 mouse, show less than a twofold increase in binding ssDNA relative to R4A. The remainder

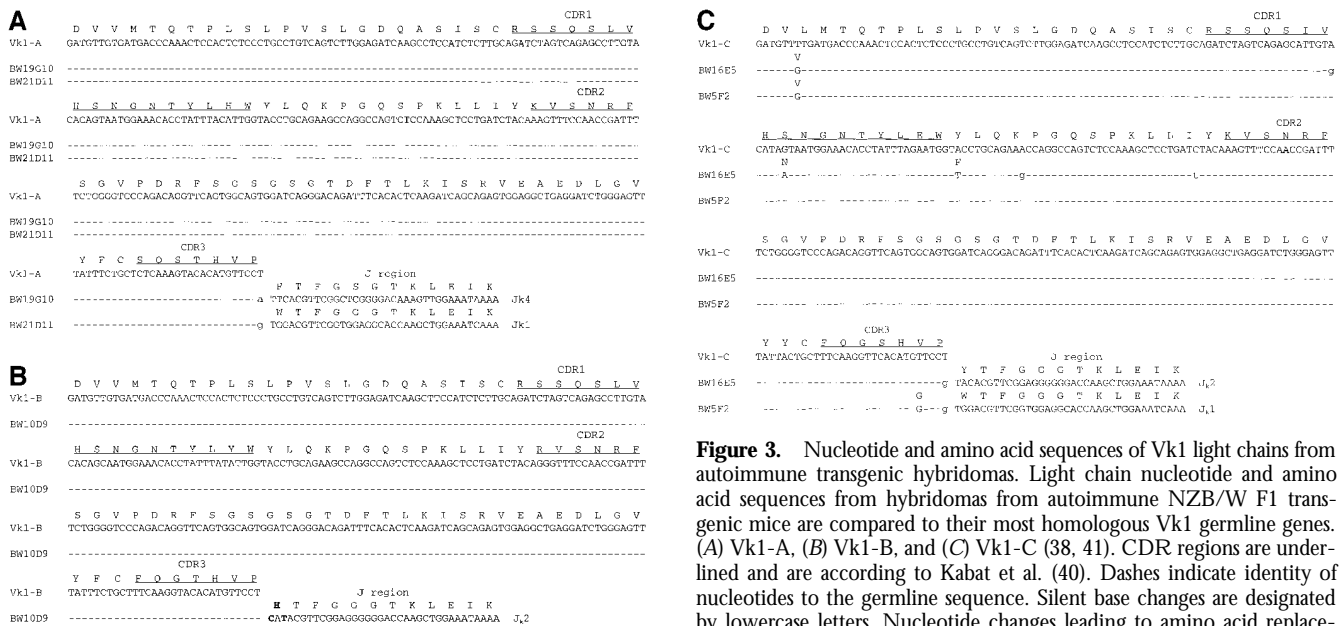
of the anti-dsDNA antibodies from both autoimmune and nonautoimmune mice display binding to ssDNA less than twofold greater than the binding of R4A.

To test for pathogenicity, a Vk1-expressing cell line, NZW145-D2, derived from an NZW transgenic mouse, and a non-Vk1 expressing line, BW7E6H1, derived from an NZB/W F1 transgenic mouse, were injected into SCID mice. NZW145D-2 and BW7E6H1 were selected for pathogenicity studies because both cell lines are allelically excluded and secrete only the transgenic antibody. Furthermore, antibodies from both hybridomas have similar binding characteristics. They both bind moderately well to dsDNA by ELISA (Fig. 5) and both have dissociation constants in the range of  $10^{-9}$ – $10^{-8}$  as detected by a filter binding assay (21). After 3 wk, kidneys were removed from these mice and stained for antibody deposition. In both cases, deposition of IgG was observed in glomeruli (Fig. 6, B and C) suggesting that both Vk1 and non-Vk1 R4A anti-dsDNA antibodies have pathogenic potential.

## Discussion

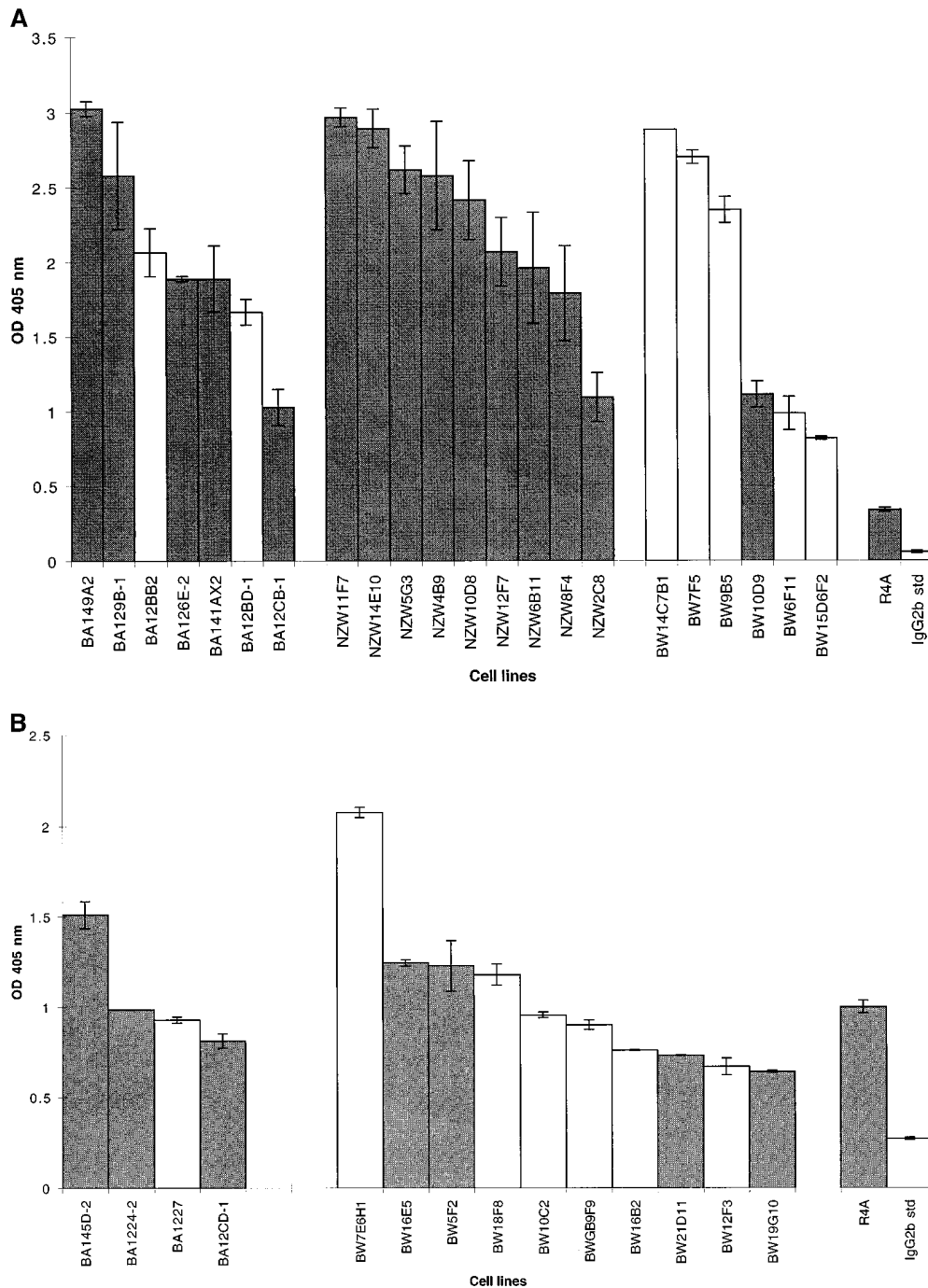
Anti-dsDNA antibodies represent a major pathogenic specificity in SLE. To understand their regulation in non-autoimmune and autoimmune hosts, we generated mice expressing the heavy chain of an IgG2b pathogenic anti-dsDNA antibody. This heavy chain, expressed in association with an endogenous light chain repertoire, can form antibodies with a variety of affinities for dsDNA. The analysis of serum and hybridomas from R4A transgenic mice leads to several important observations.

The first observation is that there is a defect in tolerance



**Figure 3.** Nucleotide and amino acid sequences of Vk1 light chains from autoimmune transgenic hybridomas. Light chain nucleotide and amino acid sequences from hybridomas from autoimmune NZB/W F1 transgenic mice are compared to their most homologous Vk1 germline genes. (A) Vk1-A, (B) Vk1-B, and (C) Vk1-C (38, 41). CDR regions are underlined and are according to Kabat et al. (40). Dashes indicate identity of nucleotides to the germline sequence. Silent base changes are designated by lowercase letters. Nucleotide changes leading to amino acid replacements in the V region are indicated by uppercase letters. Those leading to amino acid replacements in the J regions are indicated in bold. Amino acid replacements are indicated above the nucleotide sequence. Amino acid replacements from the germline J are indicated in bold. The BW prefix precedes all numeric designations of light chains derived from NZB/W F1 mice.





**Figure 5.** Binding to dsDNA. Hybridoma supernatants from BALB/c (BA), NZW, and NZB/W F1 (BW) transgenic mice were normalized to a  $\gamma$ 2b concentration of 10  $\mu$ g/ml and assayed for binding to salmon sperm dsDNA by ELISA. Wild-type R4A- $\gamma$ 2b antibody and irrelevant  $\gamma$ 2b are used as positive and negative controls, respectively. Shaded boxes, Vk1 antibodies; open boxes, non-Vk1 antibodies. (A) Strong dsDNA-binding antibodies. Strong dsDNA binders are defined as those whose ELISA measurements at 405 nm are at least fourfold greater than R4A after a 4-h incubation with alkaline phosphatase substrate solution. (B) Moderate dsDNA-binding antibodies. Moderate dsDNA binders are defined as those whose ELISA measurements at 405 nm range from 0.75–2-fold that of R4A after overnight incubation with substrate solution. Results are the average of triplicates  $\pm$  standard deviation. Standard deviations  $<0.010$  do not appear on the bar graph.

are encoded by a broad spectrum of Vk genes. This finding suggests that autoreactive B cells expressing non-Vk1 light chains are deleted from the nonautoimmune strains and that light chain usage influences cell fate. Further support for the deletion of non-Vk1-R4A B cells in nonautoimmune mice comes from studies of BALB/c mice transgenic for both R4A- $\gamma$ 2b and *bcl-2*. These mice have transgenic anti-DNA B cells in the spleen expressing a broad spectrum of Vk genes (Kuo, P., and B. Diamond, unpublished observations).

Decreased Vk1 usage in NZB/W F1 mice is not due to

an abnormality in the  $\kappa$  light chain gene locus of the NZB mouse. Autoantibody production has been observed in many different Igk-V haplotypes and RFLP analysis has failed to identify particular lupus-associated Igk-V genes (27). Previous studies suggest that the Igk-V gene loci in autoimmune mouse strains are normal (28) and Gavalchin et al. have demonstrated that the V regions encoding a set of pathogenic anti-DNA antibodies in the (NZB  $\times$  SWR)F1 lupus-prone mice are often derived from the nonautoimmune SWR parental strain (1). Allelic polymorphisms exist among individual light chain genes from different inbred

**Table 2.** Affinity Measurements of Anti-dsDNA Antibodies

Cell line	Affinity constants	Vk family
	<i>aKa</i> *	
B12CB-1	$6.60 \times 10^8$	Vk1
BA129B-1	$1.10 \times 10^9$	Vk1
NZW5G3	$1.1 \times 10^9$	Vk1
NZW14E10	$1.65 \times 10^9$	Vk1
NZW2C8	$1.32 \times 10^9$	Vk1
BW10D9	$5.08 \times 10^8$	Vk1
BW7F5	$1.76 \times 10^9$	Vk10
BW16B2	$6.60 \times 10^8$	Vk10
BW14C7B1	$1.32 \times 10^9$	Vk4/5
BW9B5F7	$5.29 \times 10^9$	Vk2

\*Apparent affinity constants (aKa) measured by inhibition ELISA according to Nieto et al. (20). Data shown are representative of a minimum of two assays done in triplicate.

strains. However, this cannot account for the dominant usage of Vk1-A light chains among anti-dsDNA antibodies obtained from transgenic BALB/c mice and their relative paucity among transgenic anti-dsDNA antibodies obtained from NZB/W F1 mice because an identical Vk1-A gene is present in both strains (29). Furthermore, it is unlikely that the dominant expression of Vk1 genes by anti-dsDNA antibodies obtained from the nonautoimmune transgenic mice is due to biased use of Vk1 genes in cells expressing the R4A- $\gamma$ 2b transgene. None of the 19 randomly selected R4A- $\gamma$ 2b-expressing, non-DNA-binding antibodies from NZW hybridomas express a Vk1 light chain (17). The predominant expression of Vk1 anti-dsDNA antibodies, therefore, demonstrates that anti-DNA B cells with non-Vk1 light chains are targeted for deletion in nonautoimmune strains.

To understand why Vk1-expressing B cells escape to the periphery and persist in an unactivated, silent state, whereas non-Vk1 B cells undergo deletion in nonautoimmune strains, we looked for differences in binding to dsDNA that might

**Table 3.** Cross-reactivity with ssDNA

Cell lines	OD 405 nm
BA129B-1	$2.468 \pm 0.203$
BA126E-2	$0.303 \pm 0.051$
BW14C7B1	$2.768 \pm 0.119$
BW9B5F7	$2.202 \pm 0.101$
BW15D6F2	$0.286 \pm 0.044$
R4A	$0.159 \pm 0.013$

ELISA measurements of anti-dsDNA antibodies that cross-react with ssDNA. Results are the average of triplicates  $\pm$  standard deviation.

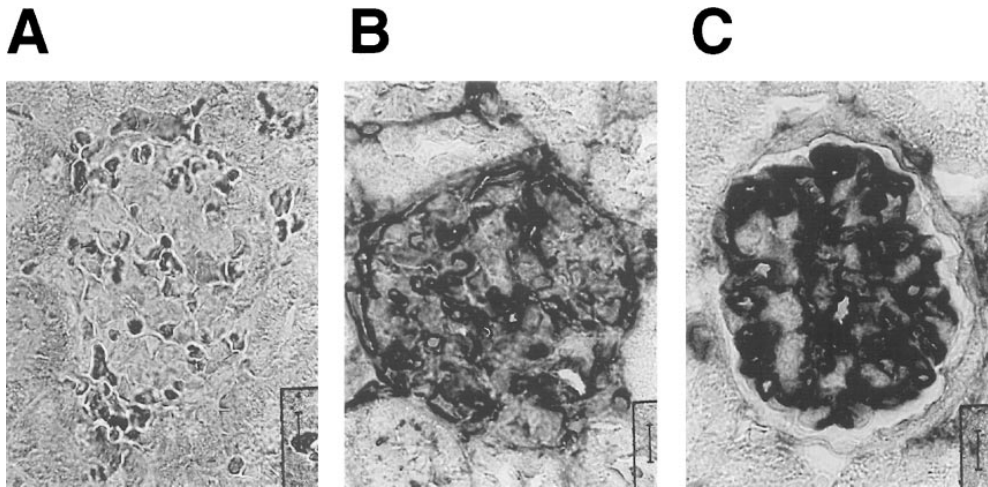
account for their differential regulation. While current models of B cell tolerance suggest that low avidity interactions with antigen lead to anergy while high avidity interactions lead to deletion, Vk1 and non-Vk1 anti-dsDNA antibodies demonstrate similar affinities for dsDNA. The difference in regulation, therefore, does not appear to be due to a difference in affinity for dsDNA. Nor does the difference in regulation correlate with differences in the pathogenic potential of these antibodies. Cell lines secreting a Vk1 and non-Vk1 anti-dsDNA antibody were injected into SCID mice and both antibodies deposited in glomeruli.

The relative contribution of kappa light chains in DNA specificity have previously been reported (30, 31). Radic et al. have demonstrated that the pairing of the 3H9 anti-DNA heavy chain with different light chains alters the ability of the antibody to bind dsDNA, cardiolipin, and RNA, although all antibodies retain the ability to bind ssDNA (31). More recently, Retter et al. has suggested that the light chain may confer upon the antibody a second cross-reactive specificity (32). We propose that perhaps it is on the basis of a second light chain-associated specificity that B cells producing R4A anti-dsDNA antibodies are either deleted or silenced. To begin to test this hypothesis, we measured binding of hybridoma antibodies to ssDNA. Although we have identified a few antibodies that bind strongly to ssDNA, we have not observed any trend that might explain the differential regulation of Vk1 and non-Vk1 antibodies. In fact, two of these antibodies use non-Vk1 genes and appear to be deleted from the nonautoimmune mice rather than anergized as one might expect.

The simplest interpretation of our studies is that DNA is not the critical antigen mediating selection of these autoreactive B cells. There is increasing evidence that anti-DNA antibodies can cross-react with several other self antigens including fibronectin, laminin, heparan sulfate, collagen, and cardiolipin, as well as nuclear antigens (33–37). If dsDNA were the critical selecting antigen and receptor occupancy alone determined cell fate, then both Vk1 and non-Vk1 B cells would undergo the same form of negative selection. Just as recent data raise the possibility that anti-DNA antibodies may mediate tissue injury by binding cross-reactive antigens, anti-DNA B cells may be regulated by cross-reactive antigens.

This transgenic model provides a unique opportunity to study the molecular basis for differential regulation of autoreactive B cells, and suggests that affinity for dsDNA is not the critical determinant of the fate of anti-dsDNA B cells. If dsDNA is indeed a self antigen capable of mediating negative selection, our data suggest that antigen binding on the membrane of a B cell may engage the B cell receptor complex to either delete or inactivate autoreactive B cells depending on properties not correlated with affinity for antigen. More likely, different light chains associating with the R4A heavy chain mediate binding to cross-reactive antigens and this cross-reactivity plays a role in determining the fate of the autoreactive anti-dsDNA B cells. The identity of these putative cross-reactive antigens remains to be determined in future studies.





**Figure 6.** Glomerular deposition of Vk1 and non-Vk1 anti-dsDNA antibodies. SCID mice were injected with  $5 \times 10^6$  hybridoma cells secreting (A) a control non-DNA-binding IgG antibody, (B) a Vk1 anti-dsDNA antibody, NZW145D-2, and (C) a non-Vk1 anti-dsDNA antibody, BW 7E6H1. Kidney sections were stained with goat anti-mouse IgG conjugated to alkaline phosphatase and viewed on a Zeiss microscope.  $\times 100$ . No deposition is observed in mice receiving the cell line producing the non-DNA binding antibody. Glomerular deposits are observed in B and C.

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