

Production of High Affinity Autoantibodies in Autoimmune New Zealand Black/New Zealand White F₁ Mice Targeted with an Anti-DNA Heavy Chain¹

Dinorah Friedmann,* Nurit Yachimovich,* Gustavo Mostoslavsky,* Yael Pewzner-Jung,* Arie Ben-Yehuda,* Klaus Rajewsky,[†] and Dan Eilat^{2*}

Lupus-prone, anti-DNA, heavy (H) chain “knock-in” mice were obtained by backcrossing C57BL/6 mice, targeted with a rearranged H chain from a V_H11(S107)-encoded anti-DNA hybridoma (D42), onto the autoimmune genetic background of New Zealand Black/New Zealand White (NZB/NZW) F₁ mice. The targeted female mice developed typical lupus serologic manifestations, with the appearance of transgenic IgM anti-DNA autoantibodies at a young age (2–3 mo) and high affinity, somatically mutated IgM and IgG anti-DNA Abs at a later age (6–7 mo). However, they did not develop clinical, lupus-associated glomerulonephritis and survived to at least 18 mo of age. L chain analysis of transgenic anti-DNA Abs derived from diseased NZB/NZW mouse hybridomas showed a very restricted repertoire of V_κ utilization, different from that of nonautoimmune (C57BL/6 × BALB/c)F₁ transgenic anti-DNA Abs. Strikingly, a single L chain was repetitively selected by most anti-DNA, transgenic NZB/NZW B cells to pair with the targeted H chain. This L chain had the same V_κ-J_κ rearrangement as that expressed by the original anti-DNA D42 hybridoma. These findings indicate that the kinetics of the autoimmune serologic manifestations are similar in wild-type and transgenic lupus-prone NZB/NZW F₁ mice and suggest that the breakdown of immunologic tolerance in these mice is associated with the preferential expansion and activation of B cell clones expressing high affinity anti-DNA H/L receptor combinations. *The Journal of Immunology*, 1999, 162: 4406–4416.

Mouse strains with inherited predisposition to autoimmune disorders provide an approach to studying the breakdown of self-immunologic tolerance and the production of autoantibodies. The F₁ hybrid cross between New Zealand Black (NZB)³ and New Zealand White (NZW) mice is considered to be the murine model most closely resembling human systemic lupus erythematosus (SLE) (1). The lupus-like disease in NZB/NZW F₁ mice is more severe in females and is accompanied by high affinity, complement fixing, mostly IgG2a and IgG2b anti-dsDNA autoantibodies that are believed to play a prominent role in the development of fatal immune glomerulonephritis in these mice (1, 2). Both NZB and NZW parents contribute multiple susceptibility genes to the immune abnormalities of the F₁ hybrid mouse (3). These include 1) increased spontaneous polyclonal B cell hyper-reactivity with elevated levels of IgM at a very early age (4, 5), 2) the appearance of IgM anti-DNA Abs at 3–5 mo of age (1, 2),

and 3) a spontaneous switch from IgM to IgG anti-DNA Abs at 5–7 mo of age concomitantly with the onset of severe SLE and renal disease (6, 7).

The role of B cell abnormalities in the development of murine SLE has been the subject of much recent investigation. Reininger et al. (8, 9) have demonstrated that SCID mice populated with NZB/NZW pre-B cells developed an SLE-like autoimmune disease with hypergammaglobulinemia, IgG anti-DNA Abs, and mild glomerulonephritis. These studies suggested that intrinsic B cell defects, inherited from both NZB and NZW parental strains, are critical for the development of murine SLE.

Anti-DNA transgenic mice are useful in vivo models for the study of self-immunologic tolerance and autoimmune disease. Most anti-DNA transgenes have been introduced to nonautoimmune mouse strains, such as BALB/c or C57BL/6, and were shown to induce B cell clonal deletion (10, 11), clonal anergy (11, 12), and receptor editing (11, 13). In only a few instances, however, have anti-DNA transgenes been backcrossed to mouse strains with autoimmune genetic background (14, 15). In these cases, the transgenic anti-DNA Abs that were recovered from autoimmune, lupus-prone mice had different properties compared with anti-DNA from normal strains. For example, the heavy (H) chain anti-DNA transgenes that were introduced to MRL/*lpr* or NZB/NZW F₁ mice were combined with different sets of endogenous light (L) chains than those used in normal transgenic mice (14, 15) to produce Abs that stained nuclei in a homogeneous nuclear fashion, characteristic of SLE (14, 16). Additionally, the transgenic anti-DNA B cells in lupus-prone mice were shown to escape tolerance induction, to undergo clonal expansion, and to be less efficient in inhibiting endogenous H chain rearrangement (allelic exclusion) on the autoimmune background (14–16).

The studies described to date suffer from a number of limitations, such as the use of a γ H chain transgene (15) that may

*Division of Medicine, Hadassah University Hospital, Faculty of Medicine, Hebrew University, Jerusalem, Israel; and [†]Institute for Genetics, University of Köln, Köln, Germany

Received for publication February 6, 1998. Accepted for publication January 19, 1999.

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

¹ This work was supported by the German-Israeli Foundation for Scientific Research and Development, the U.S.-Israel Binational Science Foundation, the Israel Science Foundation, the European Union through Grants BI02-CT94-2005 and BI04-CT96-0077, and the Deutsche Forschungsgemeinschaft through Grant SFB 243.

² Address correspondence and reprint requests to Dr. Dan Eilat, Division of Medicine, Hadassah University Hospital, P.O. Box 12000, Jerusalem 91120, Israel. E-mail address: eilatd@md2.huji.ac.il

³ Abbreviations used in this paper: NZB, New Zealand Black; NZW, New Zealand White; SLE, systemic lupus erythematosus; H chain, heavy chain; L chain, light chain; FANA, fluorescent antinuclear Ab; CDR, complementarity-determining region.

hamper the normal maturation of B cells in the bone marrow (17) and the inability of conventional Ig transgenes to undergo isotype switching, receptor editing, and affinity maturation by somatic hypermutation. These limitations, which pertain to essential steps in the development of autoimmune disease in lupus-prone mice, may be overcome by targeting the H chain transgene to the mouse Ig heavy chain locus (18). In a previous study (11) we have analyzed the various tolerance mechanisms that prevent autoimmunity in (BALB/c \times C57BL/6) F_1 mice, targeted with germline-encoded or somatically mutated anti-DNA H chain (D42). A quantitative analysis of B cell populations in the bone marrow as well as of J κ utilization and DNA binding of hybridoma Abs suggested that immature B cell deletion and L chain editing were the major mechanisms affecting tolerance. Nevertheless, autoreactive, anti-DNA B cells that showed features of clonal anergy could be recovered in the periphery of mice targeted with the somatically mutated H chain. In the present study we have backcrossed the germline-encoded D42 H chain onto the NZB genetic background and subsequently obtained lupus-prone NZB/NZW F_1 targeted mice. Transgenic IgM anti-DNA autoantibodies began to appear at a relatively young age (2–3 mo). At 6–7 mo of age, a large proportion of the targeted NZB/NZW B cells produced isotype-switched, somatically mutated, high affinity anti-DNA Abs, characteristic of fully developed SLE in these autoimmune mice.

Materials and Methods

Mice

NZB and NZW mice were purchased from Harlan (Oxon, U.K.) and were bred at the animal facility of the Hebrew University Medical School (Jerusalem, Israel). The generation of H chain-targeted gID42i mice on a C57BL/6 background was described in detail previously (11). The unmutated V_H11 -encoded, D42H transgene has been backcrossed onto the NZB background for six generations, followed by a single cross between a transgenic NZB female and a wild-type NZW male to obtain the lupus-prone NZB/NZW F_1 hybrid. Mice heterozygous for the targeted V_H gene were identified by PCR analysis of tail DNA, using D42H leader and J_H1 -specific primers, and by FACS analysis of peripheral blood lymphocytes, using a D42-specific anti-Id reagent (11). The development of overt SLE was assessed for each backcross in 6- to 8-mo-old nontransgenic littermates, using serologic (IgG anti-DNA), physiologic (proteinuria), and pathologic (immunofluorescence of kidney glomeruli) findings as major criteria (1). Proteinuria was measured by dipsticks (Albustix, Bayer Diagnostics, Basingtoke, U.K.). Clinical nephritis was defined as 2+ or greater proteinuria on at least two consecutive examinations. Values of 1+, 2+, 3+, and 4+ correspond to 0.3, 1, 3, and 20 g/l, respectively.

Immunofluorescence of cell nuclei and mouse kidney sections

Fluorescent anti-nuclear Ab (FANA) staining was conducted using permeabilized Hep-2 cells as the substrate (Kallestad, Chaska, MN) and FITC conjugates of goat anti-mouse IgM or IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). The slides were inspected by a fluorescence microscope (Zeiss, Oberkochen, Germany). Mouse kidneys were fixed in 4% paraformaldehyde for 48 h and then dehydrated with increasing concentrations (70–100%) of ethanol. The kidneys were then incubated with xylene (30 min) and embedded in paraffin. Four-micron sections were cut by a microtome and deparaffinized by heating to 65°C (20 min) and incubating twice (10 min) with xylene. The slides were then rehydrated by decreasing concentrations of ethanol and treated with 0.1% pronase (type XXIV, Sigma) for 10 min at 37°C. Staining was conducted by incubating the kidney sections with FITC-conjugated goat anti-mouse IgG for 30 min at room temperature in a humid chamber, followed by washing three times (10 min) with PBS, then counterstaining with 0.1% Evans Blue.

Other experimental procedures

Flow cytometric analysis, hybridoma production, sequence determination, and Ab binding tests (ELISA, nitrocellulose filter assay) were conducted as described previously (11). IgG mAbs were purified from serum-free, protein-free medium (Sigma, St. Louis, MO) by protein A-Sepharose (Pharmacia, Piscataway, NJ) affinity chromatography. The equilibrium DNA binding affinity of the purified Abs was determined by the nitrocellulose filter assay as described previously (19). The targeted locus genotype was

analyzed by PCR using a sense D42 leader primer and an antisense CDR3 primer as described previously (11). Sequence analysis of Ab H and L chains was performed on hybridoma cDNA by direct sequencing (11). In addition to the $V\kappa$ PCR primers used previously, the universal sense primer $V\kappa$ UP1 5'-GACATTCAGCTGACCCAGTCTCCA-3' was employed. D- J_H and V_H -D- J_H rearrangements on the untargeted H chain allele were analyzed by Southern blotting with a J_H pJ11 probe (20) and by PCR using the sense 5'-DSP2 or universal degenerate V_H primers (11) and the following anti-sense 3'- J_H primers: J_H1 , 5'-ACGGTGACCGTGGTCCCTGCGCCCCAGACA-3'; J_H2 , 5'-CTGAATAGAAGAGAGAGGTTTTAA GGACTC-3'; J_H3 , 5'-AGAGACAGTGACCAGAGTCCCTTGCCCCCA-3'; and J_H4 , 5'-GAGGAGACGGTGACTGAGGTTC-3'.

Germline configuration of the untargeted H chain allele was determined by PCR, using a sense 5'- J_H1 primer (5'-CAGATGGGCCCATCCAGTT GAGTTAAGGTG-3') and the anti-sense 3'- J_H1 primer.

Results

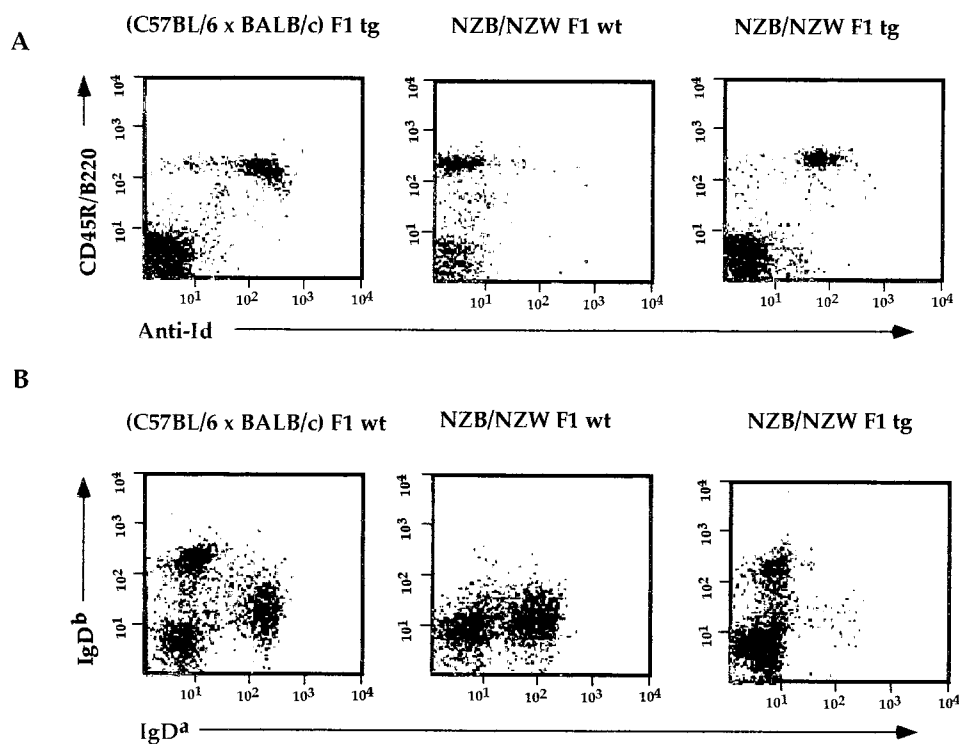
Flow cytometric analysis of B cells derived from NZB/NZW F_1 mice, targeted with the gID42 V_H gene

C57BL/6 mice targeted with the unmutated, V_H11 -encoded, D42 anti-DNA H chain (gID42i) (11) were backcrossed for six generations onto the NZB genetic background. The transgenic NZB mice were crossed once with unmanipulated NZW mice to obtain the lupus-prone NZB/NZW F_1 line. Disease manifestations (proteinuria, glomerulonephritis) were apparent in nontransgenic animals from backcross 4 onward. Backcross 5 and 6 nontransgenic littermates were indistinguishable from unmanipulated NZB/NZW F_1 mice in kidney pathology and survival. Transgenic animals were identified by PCR analysis of the targeted V_H gene and by FACS analysis of peripheral blood B cells (Fig. 1A), using a V_H11 -specific rabbit anti-idiotypic reagent (11). A two- to threefold difference in Id fluorescence intensity was observed between (C57BL/6 \times BALB/c) F_1 and NZB/NZW F_1 transgenic B cells. This may be attributed to a difference in the expressed L chain repertoires in the two mouse strains, leading to different binding affinities of the anti-Id reagent, since no clear difference in fluorescence intensity was found between the two B cell populations using a monoclonal anti-IgM reagent for FACS staining (data not shown). Allotype-specific reagents could not be employed for analyzing the targeted μ -chain, as in the case of (C57BL/6 \times BALB/c) F_1 mice (11); however, the anti- δ^b and anti- δ^a reagents were very specific for IgD of C57BL/6 and NZB/NZW mice, respectively (B. Tsao, unpublished observations). Fig. 1B shows that allelic exclusion was maintained in NZB/NZW targeted B cells, and that the proportions of transgenic (IgD b) and endogenous (IgD a) splenic B cells were very similar to those found in (C57BL/6 \times BALB/c) F_1 mice (11). The same proportions were also found in mature bone marrow B cells (not shown).

Serum anti-DNA autoantibodies in gID42 V_H -transgenic NZB/NZW mice

In sharp contrast with targeted (C57BL/6 \times BALB/c) F_1 mice, gID42 V_H -targeted NZB/NZW female mice developed an age-related anti-DNA serology that is typical of murine SLE (1, 2). At a relatively young age (2–3 mo) these mice demonstrated a significant immune response to DNA that consisted almost exclusively of IgM Abs (Fig. 2, A and B). These autoantibodies were of medium to high affinity, as suggested by their binding in the solution phase, nitrocellulose filter assay (Fig. 2C). At 6–7 mo of age, spontaneous isotype switching occurred (6, 7), and high affinity IgG anti-DNA Abs were prevalent in the serum of transgenic NZB/NZW mice (Fig. 2, B and C). Unlike the situation in (C57BL/6 \times BALB/c) F_1 mice, we could not use allotype-specific reagents to distinguish between transgenic and endogenous serum Igs in

FIGURE 1. Representative flow cytometric analysis of B cells from 2-mo-old NZB/NZW F₁ transgenic mice. *A*, Peripheral blood B cells were stained with a polyclonal rabbit anti-mouse D42 Id Ab (11), followed by FITC-F(ab')₂ goat anti-rabbit IgG for detection of Id-positive cells, and with phycoerythrin-RA3-6B2 (11) for detection of CD45R/B220. *B*, Splenocytes were stained with phycoerythrin-4/4D (a gift from Dr. Tokuhisa, Chiba, Japan) and FITC-10-4.22 (53) for detection of IgD^b and IgD^a Igs, respectively.



targeted NZB/NZW mice; however, subsequent hybridoma analysis indicated that practically all anti-DNA autoantibodies in the autoimmune mice were produced by the transgenic B cells.

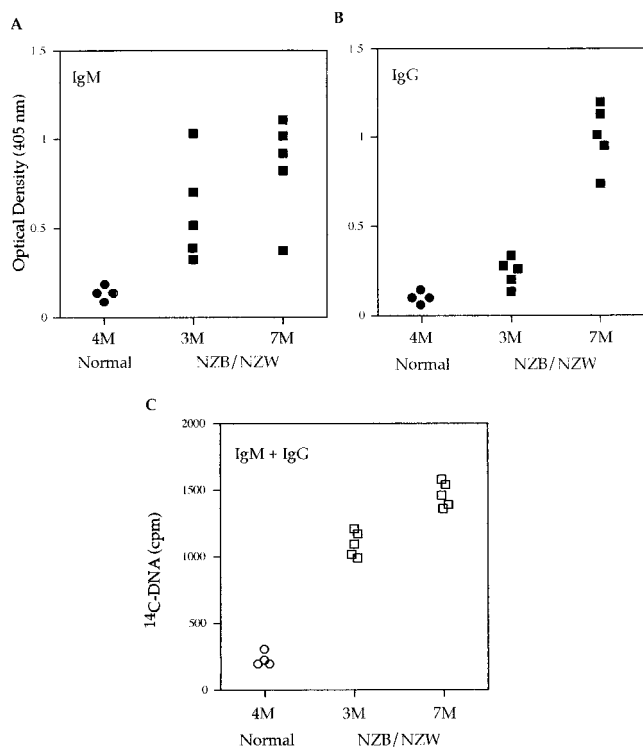


FIGURE 2. Anti-DNA serum levels in normal (C57BL/6 × BALB/c)F₁ and autoimmune NZB/NZW F₁ transgenic mice. IgM (*A*) and IgG (*B*) anti-DNA were measured by ELISA (11). *C*, IgM and IgG anti-DNA were determined by the nitrocellulose filter assay (11), using ¹⁴C-labeled *Escherichia coli* dsDNA (37 ng, 1850 cpm) with decomplexed mouse serum (10 μl). Symbols represent individual mice. Serum levels of anti-DNA Abs in normal mice remained negative at least until 12 mo of age.

Production of hybridomas secreting anti-DNA mAbs from transgenic NZB/NZW F₁ mice

Splenocytes from young (2 mo) and old (7 mo) transgenic NZB/NZW female mice were fused with NSO myeloma cells (Table I). Interestingly, polyclonal stimulation by LPS was required for the production of hybridomas from young NZB/NZW mice (Table I) as well as from normal (C57BL/6 × BALB/c)F₁ mice (11). On the contrary, hybridomas from old autoimmune mice were produced much more efficiently if no mitogenic stimulation was employed before the fusion experiment. This may reflect the different activation states of the spleen cell populations, as hybridoma production is probably dependent on B cell activation (21), and activated B cells are inhibited in LPS-induced differentiation (22). PCR analysis of hybridoma DNA using primers specific for the targeted gene showed that essentially all hybridomas derived from young mice, but only about 50% of hybridomas from old mice were of transgenic origin. The nontransgenic hybridoma Abs were mostly of the IgM class, and none of them could bind DNA in either the ELISA or the nitrocellulose filter assay. This may reflect a selective expansion in the periphery of activated, fusible B cell clones

Table I. Characterization of hybridoma clones derived from transgenic NZB/NZW F₁ mice^a

NZB/NZW Mouse	LPS ^b	IgM Clones	IgG Clones
Female, 2 mo old	+	124	0
Anti-DNA ^c		6	—
	—	0	0
Female, 7 mo old	+	8	1
Anti-DNA		1	1
	—	102	28
Anti-DNA		39	20

^a Hybridoma expressing the nontransgenic (endogenous) allele were excluded from this analysis.

^b Fusion of splenocytes was performed on day 3 after LPS activation.

^c Specificity for DNA was determined by ELISA. Optical density values of 10 times greater than background or higher were considered positive.

Table II. Heavy chain rearrangements in several anti-DNA transgenic hybridomas

Hybridoma Clone ^a	Ig Isotype	V _H	V _L	Configuration of Untargeted Allele ^b	Southern Bands ^c
a32	IgM	D42	V κ 073	gl	gl, tg
a79	IgG2a	D42	V κ 4	DJ _{H3}	gl, tg, 5.3 kb
a102	IgG2b	D42	D42	gl	gl, tg
a82	IgG2b	D42	D42	DJ _{H2}	gl, tg, 5.4 kb
a122	IgG2a	D42	D42	DJ _{H3}	gl, tg, 6.3 kb
a131	IgM	D42	D42	ND ^d	gl, tg, 4.0 kb, 4.7 kb
a134	IgM	D42	D42	DJ _{H1} , DJ _{H2}	gl, tg, 5.4 kb, 6.5 kb
b6	IgG2a	D42	D42	gl	gl, tg
b11	IgM	D42	D42	DJ _{H2}	gl, tg, 5.4 kb
b29	IgM	D42	D42	ND ^d	gl, tg, 5.0 kb
b55	IgG2b	D42	D42	DJ _{H4}	gl, tg, 3.5 kb
b16	IgG2a	D42	D42	DJ _{H2}	gl, tg, 3.8 kb
NSO ^e					gl

^a a and b refer to hybridoma clones from mouse a and b, respectively.

^b Hybridoma clones showing only germline and transgene-related bands in Southern blot and a germline PCR band with primers 5' to J_{H1} were considered to have their untargeted allele in the germline configuration. DSP2-J_H rearrangements were determined by PCR using anti-sense primers located 3' to J_{H1}, J_{H2}, J_{H3}, or J_{H4} in combination with a sense primer specific for the 5' flanking region of all DSP2 genes (11). No VDJ rearrangements were seen by PCR in transgenic hybridomas using universal V_H sense primer (11) and antisense J_{H2} or J_{H4} primers, while control hybridomas J25 (VDJ2) and 1D9 (VDJ4) showed PCR bands of the expected size. gl, germline; tg, transgene.

^c Southern blots were carried out following *Eco*RI digestion of hybridoma DNA and hybridization with a pJ11 probe (20). The sizes of the germline and the transgene hybridization bands were 6.4 and 3.2 kb, respectively.

^d ND, not determined (negative DSP2-J_H PCR).

^e Myeloma partner in the fusion experiments.

expressing the untargeted H chain allele, since the fraction (~5%) of nontransgenic, mature B cells (δ^a -positive) in bone marrow and spleen of young and old transgenic NZB/NZW mice remained constant and similar (data not shown).

None of the NZB/NZW hybridomas produced more than one Ig isotype. This complete allelic exclusion at the cellular level contrasts with results obtained with conventional transgenes in autoimmune animals (14–16) and confirms the FACS analysis of targeted B cells (Fig. 1). The maintenance of allelic exclusion in the targeted NZB/NZW B cells was also supported by Southern blotting and PCR analysis of genomic DNA obtained from a random sample of hybridoma clones (Table II). All the tested DNA samples showed at least two J_H hybridizing bands in Southern blots corresponding to one myeloma-derived germline band and one rearranged transgenic band. Additional bands, presumably corresponding to rearrangements on the untargeted H chain allele were present in nine of 12 transgenic hybridomas. However, in seven of nine DNA samples these additional rearrangements could be accounted for by D-J_H rearrangements, as revealed by PCR analyses with primers specific for 5'-DSP2 and 3'-J_H DNA sequences. The remaining two hybridomas (a131 and b29; Table II) may have rearranged non-DSP2 D segments, such as DFL16 or DQ52, as none of the 12 hybridomas showed any evidence of VDJ rearrangements on the untargeted allele in PCR reactions with a universal V_H degenerate primer and primers specific for J_{H2} or J_{H4} DNA sequences (Table II).

The hybridoma analysis (Table I) is generally compatible with the serologic results. In the absence of appropriate anti-allotypic reagents for the NZB/NZW mouse, it was not possible to prove that all anti-DNA serum Abs shown in Fig. 2 carried the transgenic allotype. However, all anti-DNA hybridoma Abs derived from the three fusion experiments shown in Table II were encoded by the H chain transgene as analyzed by PCR and nucleotide sequencing. About 5% of the 2-mo-old mouse hybridomas secreted anti-DNA IgM mAbs that were binding DNA in both the ELISA (Table I) and the nitrocellulose filter assay (not shown) and were unmutated. These Abs, like those obtained from the serum (Fig. 2), therefore had a substantially higher affinity for DNA than the transgenic anti-DNA mAbs derived from normal mice, which were com-

pletely negative in the filter assay (11). At 7 mo of age, the proportion of transgenic IgM anti-DNA hybridomas has increased to about 40%, and a significant fraction of the hybridoma population (~30%) had switched to IgG (Table I). All the transgenic IgG hybridomas secreted either IgG2a or IgG2b mAbs, and >70% of them reacted with DNA in the two binding assays (Table I and Fig. 5A). All the IgM and IgG anti-DNA mAbs were of transgenic origin, since none of the hybridomas expressing the untargeted H chain allele could bind DNA.

Properties of anti-DNA mAbs from targeted NZB/NZW mice

The nucleotide (and amino acid) sequences of representative V_H genes from hybridomas derived from two 7-mo-old transgenic NZB/NZW mice are shown in Fig. 3A. Of 22 sequenced H chain transgenes, 18 accumulated somatic mutations, and only four retained the germline sequence. Both μ and γ H chains had somatic mutations. In two of the V_H genes (e.g., b66; Fig. 3A) the recurrent Ser to Asn mutation at position 35 of CDR1 was found. This most prevalent mutation in V_{H11}-encoded anti-DNA H chains (19) increased the affinity of D42 IgM by about 10-fold (19). Interestingly, no replacement somatic mutations were found along the Arg-rich CDR3 in any of the 22 sequenced H chains. This suggests that the integrity of CDR3 is essential for high affinity DNA binding. Indeed, we have previously shown (19) that replacement of the Arg-rich CDR3 abolished DNA binding completely.

Sequence analysis of L chains from hybridomas derived from the same 7-mo-old transgenic NZB/NZW mice showed a strikingly restricted repertoire of V_L genes (Figs. 3B and 4). Seventeen of the 20 sequenced L chains had a single V_L gene (RF, 062), rearranged to a single J κ segment (J κ 5). This L chain was identical with that of the original D42 hybridoma, which had been isolated from a diseased NZB/NZW female mouse 15 yr previously (23, 24). One of the two transgenic NZB/NZW mice appeared to express this L chain exclusively, resulting in an essentially monospecific anti-DNA mouse. The majority of anti-DNA hybridomas from the second transgenic mouse also expressed the D42 V κ -J κ 5 combination, but had three additional L chains encoded by different V κ -J κ combinations (Fig. 4). In contrast to the very restricted

A

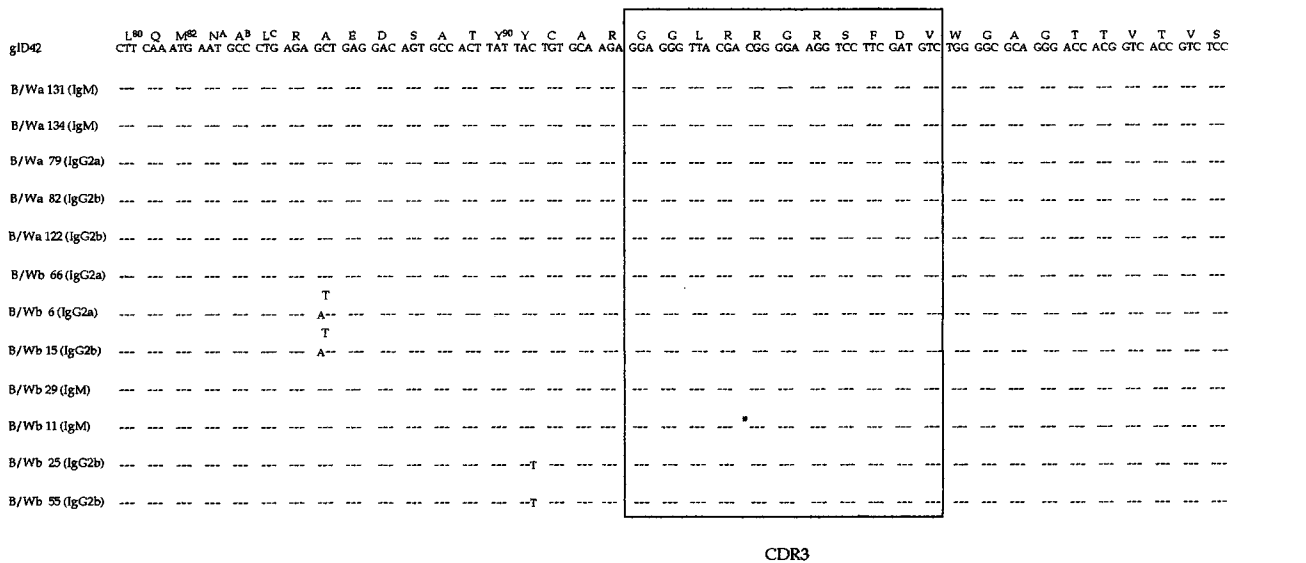
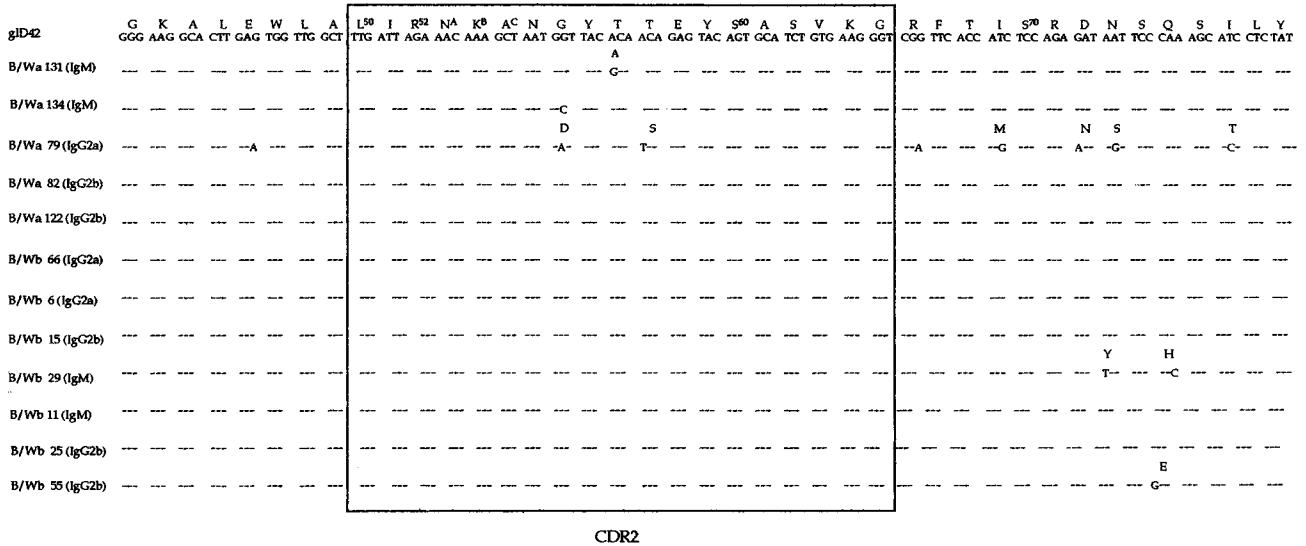
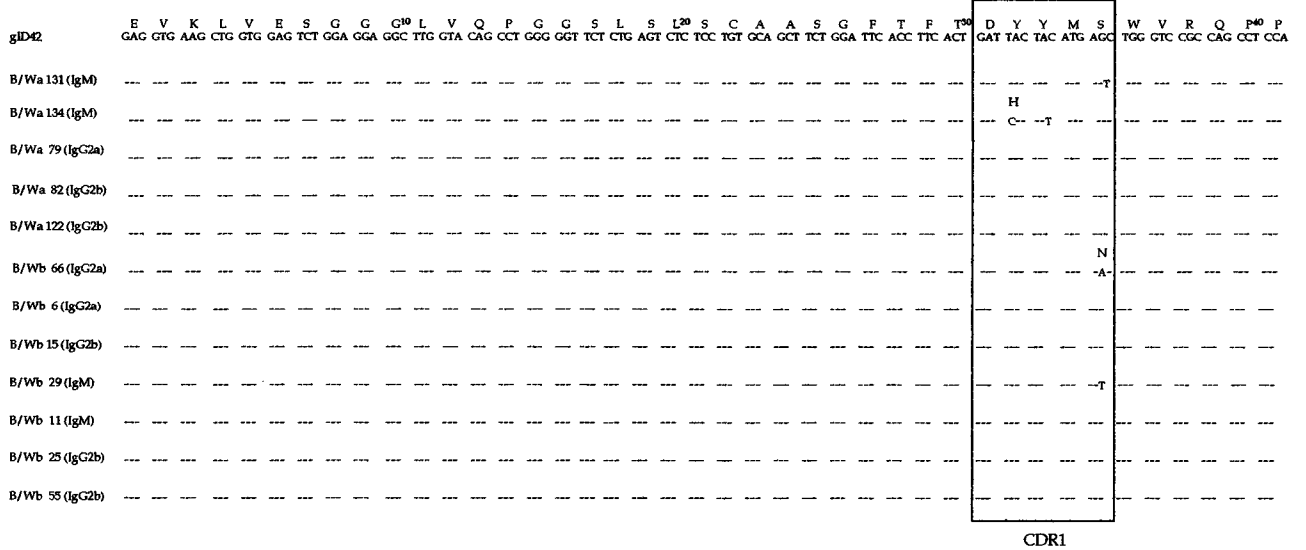


FIGURE 3. Nucleotide and deduced amino acid sequences of H (A) and L (B) chains derived from transgenic 7-mo-old NZB/NZW anti-DNA hybridomas. Dashes in sequences indicate the identities of nucleotides with residues in the *top line*. The numbering of amino acid residues is according to Kabat et al. (54). a and b refer to two individual mice.

B

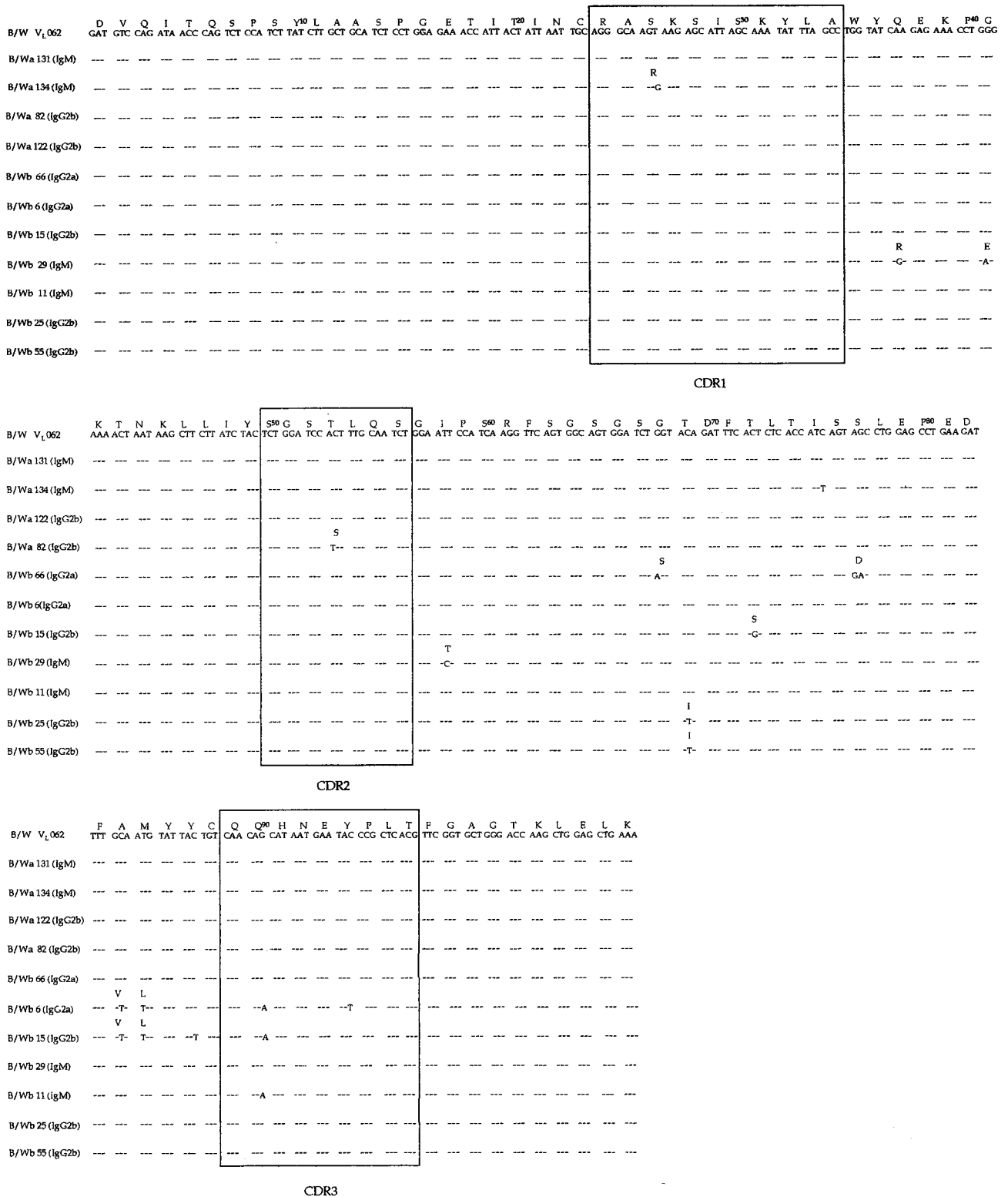


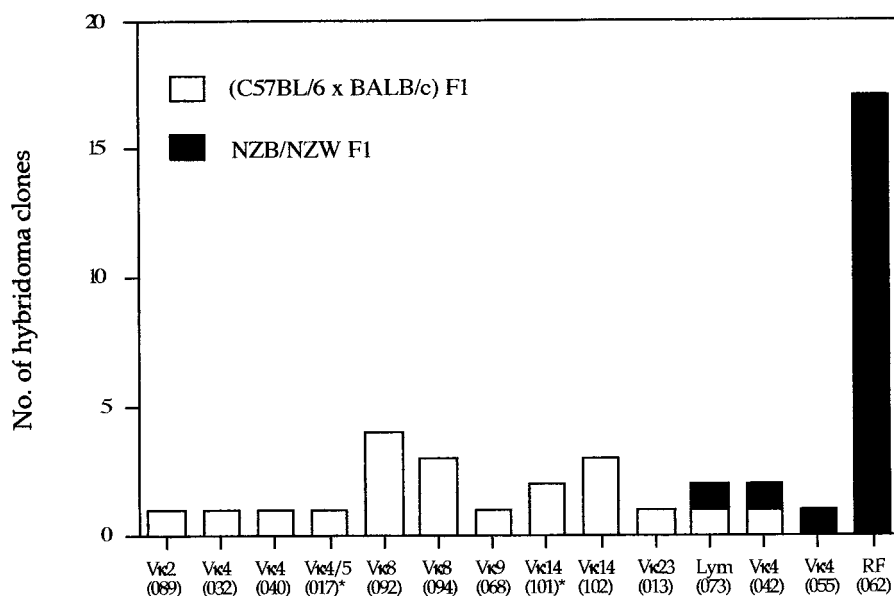
FIGURE 3. (continued)

V_κ usage in transgenic NZB/NZW mice, IgM anti-DNA transgenic hybridomas derived from D42i (C57BL/6 × BALB/c)F₁ mice (11) were much more diverse in their V_κ utilization. Twelve different V_κ genes were employed in 20 sequenced anti-DNA hybridomas (Fig. 4). There was little overlap in V_κ usage by auto-immune and normal mouse transgenic hybridomas, and the only

two V_κ genes that were common to the two groups (042 and 073; Fig. 4) were rearranged to different J_κ segments.

The anti-DNA mAbs encoded by the canonical V_HD42/V_LD42 gene pair could be derived from a repetitive selection of independently rearranged B cells or could represent a clonal expansion of a single B cell clone in each individual mouse. To distinguish

FIGURE 4. V κ gene utilization by V_H11 transgenic anti-DNA hybridomas, derived from (C57BL/6 × BALB/c)F₁ (11) and NZB/NZW F₁ mice. V κ genes were designated according to the method of Strohal et al. (45) and were rearranged to the following J κ segments: 089-J κ 2, 032-J κ 5, 040-J κ 2, 017-J κ 2, 092-J κ 2 or J κ 5, 094-J κ 2 or J κ 5, 068-J κ 5, 101-J κ 5, 102-J κ 4 or J κ 5, 013-J κ 2, 073-J κ 4 (normal) and J κ 1 (NZB/NZW), 042-J κ 5 (normal) and J κ 2 (NZB/NZW), 055-J κ 2, and 062-J κ 5. An asterisk denotes that the prototype sequence was closest to but not identical with the sequenced L chain.



between these possibilities, we have analyzed by PCR the D-J_H rearrangements of the untargeted, unexpressed H chain alleles in genomic DNA from a random sample of anti-DNA hybridoma clones (Table II). Strikingly, at least four of five D42H/D42L chain expressing hybridomas from each of the two mice showed different D-J rearrangements at the endogenous allele. This analysis suggests very strongly that multiple independent B cell clones used the same V κ -J κ gene rearrangement to pair with the transgenic H chain and were highly selected for the anti-DNA immune response in these targeted mice.

Similarly to the NZB/NZW transgenic H chains, many of the corresponding L chains were found to be somatically mutated (Fig. 3B). Interestingly, none of the replacement mutations in V_L062 (V κ D42) was present in CDRs. This may suggest that L chain somatic hypermutation does not play a major role in the affinity maturation of these anti-DNA Abs; alternatively, it is possible that framework region amino acids may increase DNA binding affinity directly or indirectly (e.g., the Ala to Val mutation at position 84 of framework region 3 in b6 and b15 L chains was also present in the original D42 L chain) (19).

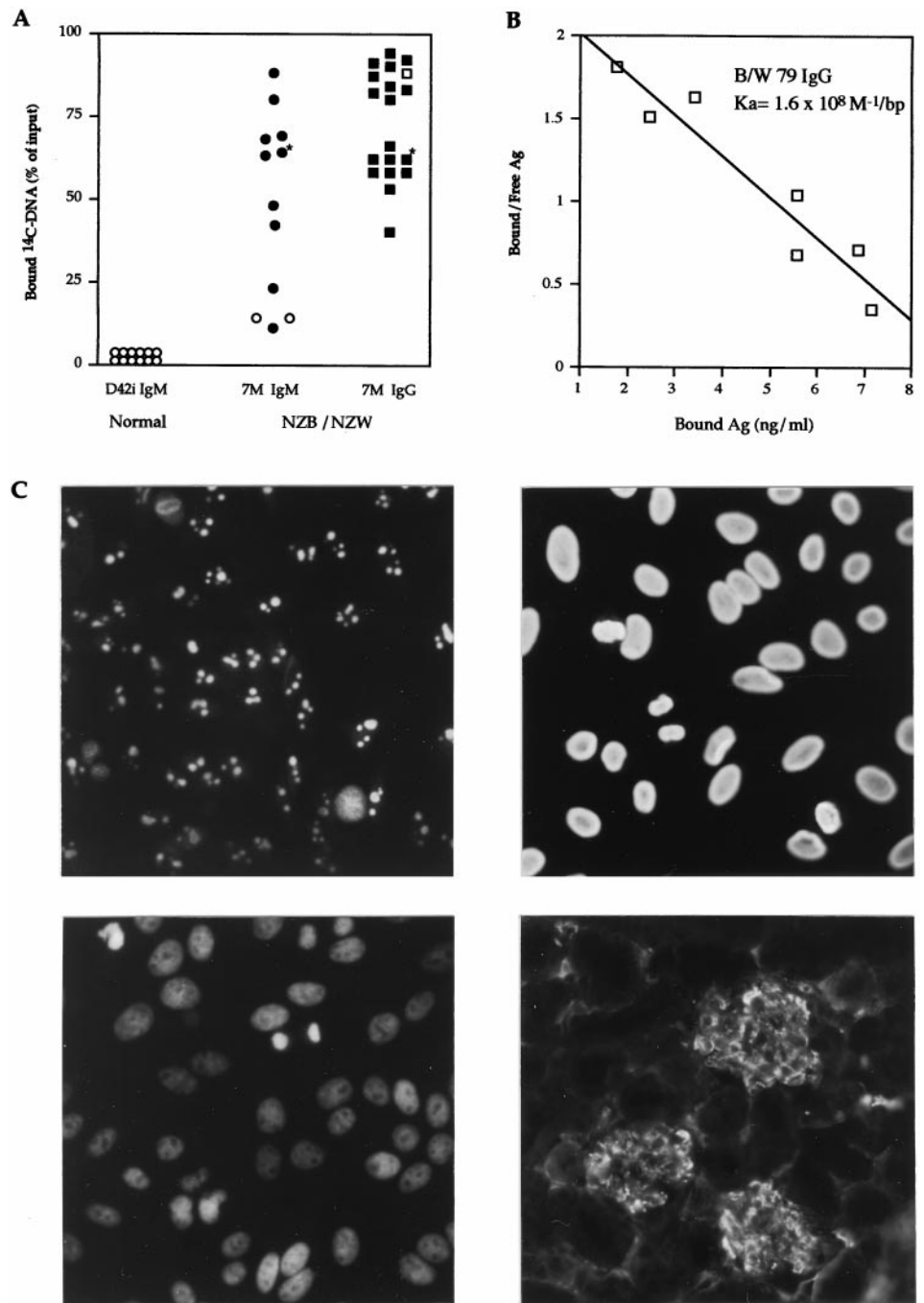
The DNA binding capacity of IgM and IgG transgenic anti-DNA Abs was evaluated by surveying a large number of hybridoma supernatants in the nitrocellulose filter assay (Fig. 5A). In hybridomas derived from 7-mo-old NZB/NZW mice, the great majority of transgenic anti-DNA Abs, particularly IgM and IgG derived from the germline encoded or somatically mutated V_HD42/V_LD42 chain combination, bound 50–90% of input radioactive DNA, suggesting a very high DNA binding affinity. In comparison, transgenic IgM anti-DNA Abs from (C57BL/6 × BALB/c)F₁ D42 targeted mice, although positive in the ELISA (11), were completely negative in the filter assay (Fig. 5A). This substantial difference in DNA binding capacity between canonical and non-canonical H/L chain combinations suggests an important role for the V κ D42-encoded L chain in generating high affinity anti-DNA autoantibodies. However, a few noncanonical H/L combinations, represented by the V_HD42/V κ 4 (055)-encoded a79 IgG mAb (Fig. 5B) had a very high binding affinity for DNA (for comparison, the apparent association constants of D42 IgG and another prototypic NZB/NZW anti-DNA Ab, A52 IgG are 5.4×10^7 and 1.3×10^8 M⁻¹/bp, respectively) (25).

The difference in DNA binding between transgenic mAbs derived from targeted normal mice (11) and those obtained from targeted NZB/NZW mice is also reflected in the pattern and intensity of nuclear staining in the fluorescent anti-nuclear Ab (FANA) test (Fig. 5C). While (C57BL/6 × BALB/c)F₁-derived IgM mAbs gave a nucleolar staining of Hep-2 cell nuclei, all NZB/NZW IgM mAbs gave a stronger, speckled nuclear staining, and all NZB/NZW IgG mAbs stained nuclei very intensely with a homogeneous pattern. The latter ANA pattern closely resembles the homogeneous nuclear fluorescent pattern obtained by Roark et al. (14) with transgenic anti-DNA mAbs from MRL/*lpr* mice. Finally, immunofluorescent staining of kidney sections derived from 7-mo-old NZB/NZW transgenic mouse showed a typical “wire loop” staining of the glomerular capillary wall with a mesangial deposition of IgG (Fig. 5C). However, the transgenic mice did not develop proteinuria above 0.3 g/L (+1), and their survival was increased to at least 18 mo of age. Of the D42 targeted mice not used in our experiments, all seven were alive at 18 mo of age, while all of their nontransgenic littermates died before reaching the age of 1 yr.

Discussion

Mouse models of autoimmunity targeted with distinct combinations of Ig or TCR chains provide an excellent opportunity for studying the genetic and immunologic factors that contribute to the loss of self tolerance and the development of autoimmune disease. In the case of SLE, a very diverse and multiclonal immune response to DNA that involves only a small fraction of the mouse B cells and serum Igs may be reduced to a nearly homogeneous immune response that can be analyzed in precise detail. For example, the V_H11(S107) gene, which is one of the most recurrent V_H genes in the murine autoimmune response to DNA in NZB/NZW mice, is found in only about 5% of anti-DNA hybridomas derived from these mice (26). This makes a detailed study of clonal activation, class switching, and somatic mutation much easier in the targeted model than in the wild-type animal. A quantitative assessment of B cell deletion and of B cell receptor editing is virtually impossible in wild-type animals because the number of cells affected by these tolerance mechanisms is very small. Additionally, the distinct and well-defined changes in the properties of

FIGURE 5. Properties of transgenic anti-DNA mAbs from NZB/NZW mice. *A*, DNA binding capacity of transgenic anti-DNA hybridoma supernatants (10 μ l, normalized to 10 μ g Ig/ml) from D42i (C57BL/6 \times BALB/c) F_1 (11) and autoimmune NZB/NZW F_1 mice. Binding is expressed as a percentage of the input [14 C]dsDNA (37 ng, 1850 cpm). Filled and empty symbols represent canonical and noncanonical H/L chain combinations, respectively. An asterisk denotes the germline sequence of both chains. *B*, Equilibrium affinity measurement in solution of a noncanonical IgG anti-DNA mAb from a 7-mo-old NZB/NZW transgenic hybridoma. Apparent affinity constants were determined as described previously (19, 25). *C*, Immunofluorescence staining of Hep-2 nuclei and NZB/NZW kidney glomeruli. *Upper left*, FANA staining with a transgenic (C57BL/6 \times BALB/c) F_1 IgM anti-DNA mAb (11); *lower left*, FANA staining with a transgenic NZB/NZW IgM anti-DNA mAb; *upper right*, FANA staining with a transgenic NZB/NZW IgG anti-DNA mAb; *lower right*, immunofluorescence of a kidney section from a 7-mo-old transgenic NZB/NZW mouse.



a single Ig gene when transferred from a normal to an autoimmune genetic background may serve as a convenient readout system for probing suspected non-Ig genes that may be involved in autoimmunity. This can be done by simple backcrossing to recombinant, transgenic, or knockout mice with defined genotypes and may apply, for example, to the recently identified disease susceptibility genes in NZB/NZW mice (27–29) or to signal transduction-modulating genes, such as CD22 (30) or Lyn (31). The study of glomerulonephritis and other Ab-dependent pathologic mechanisms in SLE may also benefit greatly from the availability of targeted mice producing well-defined autoantibodies, since the available models for pathologic studies in vivo mostly rely on the administration of malignant hybridomas to immunocompromised mice (32). The limited survival of the inoculated animals does not permit a chronic process of tissue injury that is typical of murine and human SLE.

In this study we have been able to reproduce many serologic and some pathologic features of murine SLE by targeting a single anti-DNA H chain to the genome of the NZB/NZW F_1 female mouse. In the context of the still poorly defined genetic background of this autoimmune strain, the targeted gID42 H chain gave rise to high affinity anti-DNA autoantibodies that were missing from the repertoire of (C57BL/6 \times BALB/c) F_1 mice targeted with the same rearranged V_H gene (11). Furthermore, many of these anti-DNA Abs followed a typical course of age-related disease development by switching their IgM isotype to IgG2a or IgG2b and by accumulating somatic mutations (including some that are known to increase DNA binding affinity). These results indicate that the kinetics of autoimmune disease development are similar in wild-type and transgenic lupus-prone NZB/NZW mice and that targeted mice may serve as reliable models for studying the mechanisms of autoimmunity.

Several studies have addressed the issue of allelic exclusion in B cells of autoimmune mice (14–16, 33, 34). While there are no data indicating that H chain allelic exclusion is in any way defective in nontransgenic MRL/*lpr* B cells, including the autoantibody-producing B cells (34), studies in MRL/*lpr* (14, 16) and NZB/NZW anti-DNA transgenic mice (33) have documented frequent H chain allelic inclusion among the autoantibody-producing hybridomas and suggested that allelic inclusion protects anti-DNA specific B cells from central deletion. Some of the reported results could be attributed to the difference between a targeted and a conventional H chain transgene, particularly an IgG H chain transgene, which may need to be rescued by allelic inclusion for the proper B cell development (17). The results presented in this paper do not support allelic inclusion in NZB/NZW anti-DNA B cells for the following reasons: 1) FACS analysis of knock-in heterozygous NZB/NZW B cells carrying one targeted and one untargeted H chain allele with allotype-specific reagents (Fig. 1B) showed only single allotype-expressing cell populations; 2) ELISA assays of >300 hybridomas obtained from these mice did not reveal simultaneous secretion of IgM and IgG or different IgG subclasses by individual hybridoma clones; 3) PCR analysis of several hybridomas with D_H-, J_H-, and V_H-specific primers showed a high frequency of unrearranged and incompletely rearranged configurations of the untargeted, unexpressed H chain allele, indicating efficient allelic exclusion by the V_HD42 transgene. Taken together, these results suggest that allelic inclusion does not play a major role in this model of autoimmunity.

The structural and functional properties of transgenic anti-DNA Abs, as analyzed by H/L chain pairing, solid and solution phase binding assays, and nuclear staining patterns, were very different in lupus-prone NZB/NZW mice and normal (C57BL/6 × BALB/c)_{F1} mice. It is important to point out that the NZB/NZW hybridomas and the normal mouse hybridomas were derived by different activation mechanisms, i.e., spontaneous activation in NZB/NZW mice (Table I) and LPS in normal mice (11). In several fusion experiments we observed that pretreatment of spleen cells with LPS was necessary for obtaining anti-DNA hybridomas from D42i (C57BL/6 × BALB/c)_{F1} transgenic mice, but the same treatment was counterproductive in the case of 7-mo-old NZB/NZW transgenic mice (Table I). This finding is best explained by the anergic state of the DNA-specific B cells in normal mice, as shown by several criteria (11) and by the apparent spontaneous activation of the Ab-secreting B cells in old autoimmune animals (Fig. 2). In the latter case, LPS stimulation of mature, activated B cells may be suppressed (22, 35) and/or may lead to deletion by apoptosis. Although LPS stimulation and spontaneous activation may select different populations of DNA-reactive B cells, it is likely that the hybridomas analyzed for H/L chain pairing (Fig. 4) are representative of the total anti-DNA B cell population in normal and autoimmune transgenic mice. This is because both spontaneous activation and mitogenic stimulation were repeatedly employed for B cells derived from both strains of mice. However, it cannot be excluded that other methods of activation, such as CD40 ligand and IL-4 stimulation, would yield additional populations of anti-DNA-secreting B cells. For the purpose of correlating H/L pairing chain combination with DNA binding affinity of Abs derived from normal and autoimmune mice (Fig. 5A), the route of B cell activation is less relevant, since the binding properties only depend on the particular sequences of the H/L pairs.

Although most of the transgenic anti-DNA mAbs obtained from normal mouse hybridomas were encoded by the somatically mutated D42 V_H gene (11), potentially leading to higher affinity Abs, their DNA binding capacity was substantially lower than that of the V_H D42-encoded 7-mo-old NZB/NZW mAbs (Fig. 5A). Fur-

thermore, several IgM and IgG anti-DNA Abs from 7-mo-old NZB/NZW mice whose H and L chains were unmutated bound DNA with relatively high affinity (Fig. 5A) (25), typical of anti-DNA Abs from diseased NZB/NZW mice. These results indicate that NZB/NZW mice differ from normal mice in their ability to maintain and expand B cells carrying BCR H/L chain combinations with high affinity for DNA. In the context of H chain transgenic mice, this implies that normal and autoimmune mice use different sets of L chains for pairing with the H chain transgene. This indeed was found to be the case in this study (Fig. 4) and in the study by Roark et al. (14), using a conventional anti-DNA H chain transgenic MRL mice. Furthermore, this analysis indicates that nonsomatically mutated autoantibodies may bind DNA with high affinity. In support of this conclusion, our previous analysis of the structural elements controlling anti-DNA Ab affinity has shown that the major contribution to DNA binding affinity is provided by the nonmutated rearranged configuration of the autoantibody and depends on the particular H/L chain pairing (19).

The finding that over 80% of the transgenic anti-DNA hybridomas derived from diseased NZB/NZW mice had L chains encoded by a single V_κ-J_κ combination and that many of these hybridomas represent repetitively selected, independent B cell clones was striking and unexpected. This is particularly since the V_H11-encoded H chain has been reported to combine with many different L chains to give anti-DNA specificity (summarized in Ref. 11). Several nonmutually exclusive explanations may be provided for this very restricted anti-DNA immune response. 1) The dominance of this particular H/L chain combination could result from an Ag-independent preferential association that is driven by structural properties. Although V_H and V_κ gene families have been shown to pair without bias, as would be expected from random association (36), some influence of the highly positively charged CDR3 peptide of the D42 H chain could modulate the selection of L chains for efficient H/L pairing. 2) The strong selection for the V_HD42/V_LD42 chain combination could represent a unique fine specificity for the inducing (auto) Ag. Studies on anti-histone and anti-nucleosome autoantibodies in lupus-prone mice (37, 38) as well as studies of T cells and TCRs involved in the anti-DNA autoimmune response (39) suggest that a DNA-protein, possibly a DNA-histone complex may serve as the immunizing agent, leading to anti-DNA B cell activation. Consequently, the D42 Ab binding site may be able to accommodate a partial protein epitope in addition to DNA. Weigert and his co-workers further suggested that the L chains of anti-DNA autoantibodies may be responsible for the recognition of nuclear protein and for modulating the nuclear staining patterns of anti-DNA autoantibodies (40, 41). Indeed, the different ANA patterns of transgenic Abs obtained from (C57BL/6 × BALB/c)_{F1} and 7-mo-old NZB/NZW mice (Fig. 5C) are compatible with this interpretation. 3) Anti-DNA B cell clones having this particular BCR H/L chain combination may be preferentially expanded in peripheral organs due to their particularly high affinity for the DNA autoantigen. This is suggested by direct affinity measurements in solution of D42 IgM and IgG (19, 25) and by the high DNA binding capacity of canonical IgM and IgG transgenic Abs compared with that of noncanonical anti-DNA Abs (Fig. 5A). In this regard, it is interesting to note that the few anti-DNA Abs derived from 3-mo-old transgenic NZB/NZW mice had an intermediate DNA binding capacity (20–30% of input DNA in the filter assay), and none of them was encoded by the V_HD42/V_LD42 chain combination. Although the sample size is small, this may suggest, as in the case of the immune response to the oxazolone hapten (42), that the initial immune response to DNA in NZB/NZW mice is characterized by a nonoptimal H/L chain combination with a rather low affinity, later replaced by a restricted pair of germline

H/L combinations with good affinity that further improve by somatic mutation. The homogeneous ANA fluorescent pattern of the high affinity Abs may then be explained by their ability to bind less accessible DNA structures in the nucleus.

The restricted H/L chain pairing of the transgenic anti-DNA Ig population is particularly striking when the number of independently selected B cell clones is considered. A partial analysis of hybridomas from an additional old NZB/NZW transgenic mouse has shown an overwhelming dominance (10 of 11 mAb) of the V_HD42/V_LD42 chain combination in the anti-DNA Ab population (N. Yachimovich, G. Mostoslavsky, and D. Eilat, unpublished observations). Within individual mice, several Abs bearing this H/L pair were shown to represent independent B cell clones (Table II). Furthermore, inspection of somatic mutations in both H and L sequenced chains of >20 canonical mAb convincingly showed only two pairs of clonally related hybridomas (b6; b15 and b25; b55; Fig. 3). This suggests that the autoimmune response to DNA is multiclonal. The same conclusion was reached following our detailed study of another anti-DNA-associated V_H gene (V_HBW-16) in nontransgenic NZB/NZW mice (25). This study has shown that a single V_H gene may encode a diverse population of anti-DNA B cell clones in an individual diseased animal.

The D42 V_κ-gene segment was first identified by Kofler et al. (43) in a L chain derived from an MRL mouse IgM rheumatoid factor, RF-24. The sequence could not be assigned to any of the existing V_κ groups by the classification of Potter et al. (44) or that of Strohal et al. (45) on the basis of sequence homology. It therefore represents a one-member V_κ family designated V_κRF (45). The germline gene, designated V_L 062 (45), was cloned by us from NZB and from NZW kidney DNA, and its coding sequence was identical in these two mouse strains (19). The L chain of the BALB/c H280-4 anti-influenza hemagglutinin hybridoma is also encoded by the V_L 062 gene in its rearranged germline configuration (W. Gerhard and A. J. Caton, GenBank accession no. M64167).

The site and stage at which B cell tolerance to DNA and other nuclear antigens is lost in lupus-prone mice remain unknown. Rubio et al. (34) found that B cell tolerance in anti-MHC class I transgenic MRL/*lpr* mice was substantially intact. However, the mice developed characteristic disease traits, including elevated levels of IgG anti-DNA autoantibodies. Recently, the same group reported on efficient peripheral clonal elimination of B lymphocytes in MRL/*lpr* mice bearing autoantibody transgenes (46). They suggested that peripheral tolerance in these mice is not globally defective, but that certain B cells with receptors specific for nuclear Ags are regulated differently than are cells reactive to membrane autoantigens. Our preliminary experiments, in which we have looked at the ratio of immature B to pre-B cell compartments (11) in the bone marrow of young (3 mo) and old (7 mo) NZB/NZW F₁ H chain transgenic mice support these results and suggest that there is no general defect in central B cell tolerance in these autoimmune mice, and that NZB/NZW mice do not demonstrate a diminished capacity to delete anti-DNA transgenic immature B cells compared with (C57BL/6 × BALB/c)F₁ targeted mice. This would further suggest that the escape of high affinity, anti-DNA B cell clones from the bone marrow to the periphery is a very selective process that involves a relatively small number of B cell clones. These clones may be rescued by a powerful, yet unexplained activation process during the reversible stage of immature B cell developmental arrest as suggested by Hartley et al. (47). In the periphery, this activation process would prevent the induction of B cell anergy and induce secondary response features, such as class switching and somatic mutation, that probably require the participation of autoreactive T cells (39).

The selective escape from deletion of high affinity autoreactive B cells may also be facilitated by a malfunctioning mechanism of receptor editing, that may be responsible for lowering the affinity of these autoreactive cells through L chain secondary rearrangements (11). A partial failure of this mechanism may result in a leak of autoreactive B cells to the periphery due to an overloaded system of central tolerance. An additional explanation for the presence of DNA-reactive B cells in the circulation of mice with functional central tolerance may be based on the recently demonstrated B cell receptor editing in germinal centers of peripheral organs (48, 49). This opens the interesting possibility that B cells expressing autoreactive H/L chain combinations may originate exclusively in the periphery. In this case, the failure or absence of peripheral tolerance could account for the autoimmune manifestations of lupus-prone mice in the absence of any impairment in central tolerance.

It is interesting to note that despite a clear, disease-related anti-DNA serology demonstrated by the targeted NZB/NZW mice and the characteristic glomerular staining pattern (Fig. 5C), these mice did not develop severe proteinuria like their nontransgenic littermates and were alive at 18 mo of age. This unusual pathology is somewhat similar to that reported recently by Clynes et al. (50) in Fc receptor γ chain-deficient NZB/NZW mice. One possible explanation for the absence of severe nephritis may be the absence of a polyclonal anti-nuclear immune response. Additionally, we have consistently observed that the D42 mAb is only mildly pathogenic in an in vivo mouse model (G. Mostoslavsky and D. Eilat, unpublished observations) and in the isolated perfused rat kidney model (51). The molecular properties that distinguish pathogenic from nonpathogenic anti-DNA autoantibodies are currently not well understood and are the subject of intensive investigation (52).

References

- Hahn, B. 1993. Animal models of systemic lupus erythematosus. In *Dubois' Lupus Erythematosus*. D. Wallace and B. Hahn, eds. Lea & Febiger, Philadelphia, p. 157.
- Theofilopoulos, A. N., and F. J. Dixon. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol.* 37:269.
- Vyse, T., and B. Kotzin. 1996. Genetic basis of systemic lupus erythematosus. *Curr. Opin. Immunol.* 8:843.
- Izui, S., P. McConahey, and F. Dixon. 1978. Increased spontaneous polyclonal activation of B lymphocytes in mice with spontaneous autoimmune disease. *J. Immunol.* 121:2213.
- Klinman, D. M., and A. D. Steinberg. 1987. Systemic autoimmune disease arises from polyclonal B cell activation. *J. Exp. Med.* 165:1755.
- Steward, M., and F. Hay. 1976. Changes in immunoglobulin class and subclasses of anti-DNA antibodies with increasing age in NZB/NZW F₁ hybrid mice. *Clin. Exp. Immunol.* 26:363.
- Papoian, R., R. Pillarsetty, and N. Talal. 1977. Immunological regulation of spontaneous antibodies to DNA and RNA. II. Sequential switch from IgM to IgG in NZB/NZW F₁ mice. *Immunology* 32:75.
- Reininger, L., T. Radaszkiewicz, M. Kosco, F. Melchers, and A. G. Rolink. 1992. Development of autoimmune disease in SCID mice populated with long-term 'in vitro' proliferating (NZB × NZW)F₁ pre-B cells. *J. Exp. Med.* 176:1343.
- Reininger, L., T. H. Winkler, C. P. Kalberer, M. Jourdan, F. Melchers, and A. G. Rolink. 1996. Intrinsic B cell defects in NZB and NZW mice contribute to systemic lupus erythematosus in (NZB × NZW)F₁ mice. *J. Exp. Med.* 184:853.
- Chen, C., Z. Nagy, M. Z. Radic, R. R. Hardy, D. Huszar, S. A. Camper, and M. Weigert. 1995. The site and stage of anti-DNA B-cell deletion. *Nature* 373:252.
- Pewzner-Jung, Y., D. Friedmann, E. Sonoda, S. Jung, K. Rajewsky, and D. Eilat. 1998. B cell deletion, anergy and receptor editing in "knock in" mice targeted with a germline-encoded or somatically mutated anti-DNA heavy chain. *J. Immunol.* 161:4634.
- Tsao, B. P., A. Chow, H. Cheroutre, Y. W. Song, M. E. McGrath, and M. Kronenberg. 1993. B cells are anergic in transgenic mice that express IgM anti-DNA antibodies. *Eur. J. Immunol.* 23:2332.
- Radic, M. Z., J. Erikson, S. Litwin, and M. Weigert. 1993. B lymphocytes may escape tolerance by revising their antigen receptors. *J. Exp. Med.* 177:1165.
- Roark, J., C. Kuntz, K. Nguyen, A. Caton, and J. Erikson. 1995. Breakdown of B cell tolerance in a mouse model of systemic lupus erythematosus. *J. Exp. Med.* 181:1157.
- Spatz, L., V. Saenko, A. Iliev, L. G. Jones, and B. Diamond. 1997. Light chain usage in anti-double-stranded DNA B cell subsets: role in cell fate determination. *J. Exp. Med.* 185:1317.

16. Roark, J., C. Kuntz, K.-A. Nguyen, L. Mandik, M. Cattermole, and J. Erikson. 1995. B cell selection and allelic exclusion of an anti-DNA Ig transgene in MRL-*lpr/lpr* mice. *J. Immunol.* 154:4444.
17. Kurtz, B., P. Witte, and U. Storb. 1997. $\gamma 2b$ provides only some of the signals normally given via μ in B cell development. *Int. Immunol.* 9:415.
18. Taki, S., M. Meiering, and K. Rajewsky. 1993. Targeted insertion of a variable region gene into the immunoglobulin heavy chain locus. *Science* 262:1268.
19. Pewzner-Jung, Y., T. Simon, and D. Eilat. 1996. Structural elements controlling anti-DNA antibody affinity and their relationship to anti-phosphorylcholine activity. *J. Immunol.* 156:3065.
20. Marcu, K. B., J. Banerji, N. A. Penncavage, R. Lang, and N. Arnheim. 1980. 5' flanking region of immunoglobulin heavy chain constant region genes display length heterogeneity in germ lines of inbred mouse strain. *Cell* 22:187.
21. Köhler, G., and C. Milstein. 1976. Derivation of specific antibody producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511.
22. Kearny, J. F., M. D. Cooper, and A. R. Lawton. 1976. B lymphocyte differentiation induced by lypopolysaccharide. III. Suppression of B cell maturation by anti-mouse immunoglobulin antibodies. *J. Immunol.* 116:1664.
23. Eilat, D., M. Hochberg, J. Pumphrey, and S. Rudikoff. 1984. Monoclonal antibodies to DNA and RNA from NZB/NZW F₁ mice: antigenic specificities and NH₂ terminal amino acid sequences. *J. Immunol.* 133:489.
24. 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.
25. Ash-Lerner, A., M. Ginsberg-Strauss, Y. Pewzner-Jung, D. D. Desai, T. N. Marion, and D. Eilat. 1997. Expression of an anti-DNA associated V_H gene in immunized and autoimmune mice. *J. Immunol.* 159:1508.
26. Tillman, D. M., N. T. Jon, R. J. Hill, and T. N. Marion. 1992. Both IgM and IgG anti-DNA antibodies are the products of clonally selective B cell stimulation in (NZB/NZW)F₁ mice. *J. Exp. Med.* 176:761.
27. Morel, L., U. H. Rudofsky, J. A. Longmate, J. Schifffenbauer, and E. K. Wakeland. 1994. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1:219.
28. Drake, C., S. Babcock, E. Palmer, and B. Kotzin. 1994. Genetic analysis of the NZB contribution to lupus-like autoimmune disease. *Proc. Natl. Acad. Sci. USA* 91:4062.
29. Kono, D. H., R. W. Burlingame, D. G. Owens, A. Kuramochi, R. S. Balderas, D. Balomenos, and A. N. Theofilopoulos. 1994. Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA* 91:10168.
30. O'Keefe, T. L., G. T. Williams, S. L. Davies, and M. S. Neuberger. 1996. Hyperresponsive B cells in CD22-deficient mice. *Science* 274:798.
31. Chan, V. W. F., F. Meng, P. Soriano, A. L. DeFranco, and C. A. Lowell. 1997. Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. *Immunity* 7:69.
32. Vlahakos, D. V., M. H. Foster, S. Adams, M. Katz, A. A. Ucci, K. J. Barrett, S. K. Datta, and M. P. Madaio. 1992. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. *Kidney Int.* 41:1690.
33. Iliiev, A., L. Spatz, S. Ray, and B. Diamond. 1994. Lack of allelic exclusion permits autoreactive B cells to escape deletion. *J. Immunol.* 153:3551.
34. Rubio, C. F., J. Kench, D. M. Russel, R. Yawger, and D. Nemazee. 1996. Analysis of central B cell tolerance in autoimmune-prone MRL/*lpr* mice bearing autoantibody transgenes. *J. Immunol.* 157:65.
35. Andersson, J., W. W. Bullock, and F. Melchers. 1974. Inhibition of mitogenic stimulation of mouse lymphocytes by anti-mouse immunoglobulin antibodies. I. Mode of action. *Eur. J. Immunol.* 4:715.
36. Kaushik, A., D. Schulze, F. Bonilla, C. Bona, and G. Kelsoe. 1990. Stochastic pairing of heavy chain and κ light-chain variable gene families occurs in polyclonally activated B cells. *Proc. Natl. Acad. Sci. USA* 87:4932.
37. Monestier, M., T. M. Fasy, M. J. Losman, K. E. Novick, and S. Muller. 1993. Structure and binding properties of monoclonal antibodies to core histones from autoimmune mice. *Mol. Immunol.* 30:1069.
38. Kramers, C., M. N. Hylkema, M. C. J. van Bruggen, R. van de Lagemaat, H. B. P. M. Dijkman, K. J. M. Assmann, R. J. T. Smeenk, and J. H. M. Berden. 1994. Anti-nucleosome antibodies complexed with nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. *J. Clin. Invest.* 94:568.
39. Kaliyaperumal, A., C. Mohan, W. Wu, and S. Datta. 1996. Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. *J. Exp. Med.* 183:2459.
40. Radic, M. Z., M. A. Mascelli, J. Erikson, H. Shan, and M. Weigert. 1991. Ig H and L chain contributions to autoimmune specificities. *J. Immunol.* 146:176.
41. Radic, M. Z., and M. Weigert. 1994. Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu. Rev. Immunol.* 12:487.
42. Berek, C., and C. Milstein. 1987. Mutation drift and repertoire shift in the maturation of the immune response. *Immunol. Rev.* 96:23.
43. Kofler, R., D. J. Noonan, R. Strohal, R. S., Balderas, N. P. H. Moller, F. J. Dixon, and A. N. Theofilopoulos. 1987. Molecular analysis of the murine lupus associated anti-self response: involvement of a large number of heavy and light chain variable region genes. *Eur. J. Immunol.* 17:91.
44. Potter, M., J. B. Newell, S. Rudikoff, and E. Haber. 1982. Classification of mouse V κ groups based on the partial amino acid sequence to the first invariant tryptophan: impact of 14 new sequences from IgG myeloma proteins. *Mol. Immunol.* 19:1619.
45. Strohal, R., A. Helmberg, G. Kroemer, and R. Kofler. 1989. Mouse V κ gene classification by nucleic acid sequence similarity. *Immunogenetics* 30:475.
46. Kench, J. A., D. M. Russel, and D. Nemazee. 1998. Efficient peripheral clonal elimination of B lymphocytes in MRL/*lpr* mice bearing autoantibody transgenes. *J. Exp. Med.* 188:909.
47. Hartley, S. B., M. P. Cooke, D. A. Fulcher, A. W. Harris, S. Cory, A. Basten, and C. C. Goodnow. 1993. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. *Cell* 72:325.
48. Papavasiliou, F., R. Casellas, H. Suh, X. F. Qin, E. Besmer, R. Pelanda, D. Nemazee, K. Rajewsky, and M. C. Nussenzweig. 1997. V(D)J recombination in mature B cells: a mechanism for altering antibody responses. *Science* 278:298.
49. Han, S., S. R. Dillon, B. Zheng, M. Shimoda, M. S. Schlissel, and G. Kelsoe. 1997. V(D)J recombinase activity in a subset of germinal center B lymphocytes. *Science* 278:301.
50. Clynes, R., C. Dumitru, and J. V. Ravetch. 1998. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 279:1052.
51. 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.
52. Hahn, B. H. 1998. Antibodies to DNA. *N. Engl. J. Med.* 338:1359.
53. Oi, V. T., and L. A. Herzenberg. 1979. Localization of murine Ig-1b and Ig-1a (IgG2a) allotypic determinants detected with monoclonal antibodies. *Mol. Immunol.* 16:1005.
54. Kabat, E. A., T. T. Wu, H. M. Perry, K. S. Gottesman, and C. Foeller. 1991. *Sequences of Proteins of Immunological Interest*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda.