Characterization of Somatically Mutated S107 V\textsubscript{H}11-encoded Anti-DNA Autoantibodies Derived from Autoimmune (NZB × NZW)\textsubscript{F1} Mice

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Summary

We have studied 19 S107 heavy chain variable region gene (V\textsubscript{H}11)-encoded monoclonal antibodies from NZBWF1 mice. These studies show that a single V\textsubscript{H} gene can encode both antibodies to foreign antigens (anti-phosphorylcholine) and to self antigens (anti-double-stranded DNA) in the same animal. All of the anti-DNA antibodies contain many somatic mutations compared with the relevant germline genes. Since the anti-DNA antibodies were extensively somatically mutated and had undergone isotype switching, the response seems to be T cell dependent. While some of the antibodies appear to be the products of an antigen-driven and antigen-selected response, a number of characteristics of the antibodies suggest that forces other than antigen are contributing to the stimulation and selection of this response.

The serum of patients with SLE contain IgG antibodies that bind strongly to double-stranded DNA (dsDNA)\textsuperscript{1} and contribute to the glomerulonephritis that is a major cause of death in this disease (2). The potential for investigating the origins of these antibodies is greatly facilitated by the availability of lupus-prone strains of mice such as (NZB × NZW)\textsubscript{F1} (NZBWFI) (3). Before the onset of detectable pathology, NZBWF1 mice produce IgM antibodies that react with DNA (4). These antibodies are usually of relatively low affinity and their V region genes have not undergone significant somatic mutation (5, 6). Since such antibodies are not associated with major pathology, they are thought to be non-pathogenic. In contrast, older autoimmune mice that have developed proteinuria and pathological changes in their kidneys have IgG anti-dsDNA antibodies in their blood and at the sites of tissue damage (3). An understanding of the molecular origins of these pathogenic autoantibodies could provide some understanding of why autoimmune lupus-prone strains of mice develop such antibodies. In particular, it would be useful to determine whether the germline genes that encode autoantibodies in autoimmune animals are different from the homologous genes in nonautoimmune mice (7), whether the same germline genes also encode antibodies against environment and self antigens, and whether the autoantibodies are specifically elicited by foreign or self antigens or are the product of a general polyclonal activation of all B cells (8).

An experimental approach to these questions was suggested by our finding that a single amino acid substitution in the S107 antiphosphorylcholine antibody converted it from an antibody that protects mice from Streptococcus pneumoniae (9) to a potentially pathogenic anti-dsDNA antibody (10). This in vitro paradigm seemed likely to be relevant to in vivo events since others had shown that S107-like anti-DNA antibodies were present in NZBWF1 and MRL/lpr mice (11, 12). Since the V\textsubscript{H}S107 germline family consists of only four highly homologous members (13), we were able to clone and sequence all of the members of this family from both NZB and NZW mice (14, 15). We also generated and sequenced five IgG anti-dsDNA antibodies encoded by the V\textsubscript{H}S107 family from an autoimmune NZBWF1 mouse and showed that they were encoded by the V\textsubscript{H}11 member of the V\textsubscript{H}S107 germline family (14).

Here we report the sequences and DNA binding properties of 14 additional antibodies from that same animal and three antibodies from another animal. These new sequences provide a better perspective of the V\textsubscript{H}11-encoded anti-DNA response. While the characteristics of some of these antibodies indicate that they are products of an antigen-driven and antigen-selected response, the sequences of others suggest that there are additional forces influencing the autoimmune response.

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\textsuperscript{1}Abbreviations used in this paper: dsDNA, double stranded DNA; FW, framework; (NZB × NZW)\textsubscript{F1}, NZBWFI; PC, phosphorylcholine; R, replacement; S, silent; ss, single stranded DNA; V\textsubscript{H}, heavy chain variable region gene.
Materials and Methods

4-mo-old female NZBWF1 mice were immunized intraperitoneally with 100 μg of PC-KLH in saline and boosted with the same antigen at 6 mo of age. Fusions were done using polyethylene glycol and the NSO myeloma cell line as a fusion partner as described previously (16, 17). Hybridomas that expressed a member of the V, S107 family were detected by RNA dot blot hybridization (18) and then cloned twice in soft agar before characterization (19).

The different V, S107 family mAbs were tested by ELISA for binding to phosphorylcholine, cardiolipin, and influenza hemagglutinin using standard techniques (20–22). The ELISA for DNA binding (23) was done using polyvinyl round-bottomed plates (Dynatech Laboratories, Inc., Alexandria, VA) which were coated with 100 μl of poly-l-lysine (10 μg/ml) and incubated for 2 h at 37°C or overnight at 4°C. Afterward, the solutions were flicked out and the plates rinsed with distilled water. DNA was diluted in 1× SSC or overnight at 4°C. The next day the plates were washed three times with PBS between and blocked with 1% BSA-PBS. For dsDNA ELISAs, calf thymus DNA filtered through nitrocellulose to remove single-stranded regions of DNA was used at a concentration of 5 μg/ml or bacteriophage λ DNA was used at 2.5 μg/ml. Commercially prepared single-stranded DNA (ssDNA) (Sigma Chemical Co., St. Louis, MO) was also used at 2.5 μg/ml. The NZBWF1 mAbs were also tested for binding to dsDNA using immunofluorescence on the microorganism Crithidia luciliae (24) using a commercially (Clinical Sciences, Whiphany, NJ) prepared Crithidia slide and rhodamine F(ab)2 rabbit anti-mouse κ chain antibody. Isoelectric focusing was done as described (25). Briefly, samples in 8 M urea were focused on a 4.5% polyacrylamide gel containing ampholines (LKB Instruments, Gaithersburg, MD) with a range of pH 3.0–10.0 and analyzed by Western blot (26).

The heavy and light chain variable regions of the S107 Vn family-encoded anti-DNA hybridomas were sequenced by dideoxynucleotide sequencing directly from the mRNA template as described by Geliebter (27) using end-labeled synthetic oligonucleotides as primers.

V, Germline Cloning. Genomic DNA extracted from the livers of NZB and NZW mice was partially digested with the restriction endonuclease Mbol and ligated into EMBL3 (28). The library was screened with a 300-bp probe derived from cloned BALB/c S107B germline DNA (29). Positive plaques were purified and rescreened with an oligonucleotide probe (5' GTGCATGTAACTTACACT3') specific for a 6-bp deletion that distinguishes the V, light chain consensus sequence of the group 2 (Table 1) antibodies from the BALB/c V, S107B germline and the group 3 V, sequences. One clone was amplified by the PCR, using primers containing unique restriction sites that facilitated forced subcloning of the amplified fragment into M13 (30, 31). Sequencing was done by the dideoxynucleotide chain-termination method using 2'-deoxyadenosine 5'-α-[3P] thiol triphosphate and M13 vectors (31).

Results

The conversion of S107 from a protective antibody to an autoantibody indicated that the V,1 germline gene, which encodes S107, could also encode anti-dsDNA antibodies (10). The only difference between S107 and its DNA binding U4 mutant is a glutamic acid to alanine substitution at residue 35 of the heavy chain variable region (20). Crystallographic studies of another V,1 encoded PC-binding antibody (32) and computer modeling of the S107 binding site (33) have demonstrated that glu 35 is a contact residue for the choline rather than the phosphate moiety of the phosphorylcholine (PC) molecule. This suggested that the glu to ala substitution might continue to allow binding to other phosphorylated molecules such as DNA. Our sequencing of the NZB and NZW germ line Vn11 genes showed that they encode a serine at residue 35 and that Vn11 was the germline gene for the one S107-like NZBWF1 antibody that had been reported (11, 34). Thus the germ line Vn11 is similar to the DNA binding mutant of Vn1 and can encode DNA binding proteins. The Vn11 gene also encodes antibodies to influenza, PC, and oxazalone in nonautoimmune strains of mice (35–40).

Taken together, these findings suggested that an examination of the antibodies encoded by the Vn S107 gene family in autoimmune mice might provide useful information on the molecular origins of anti-DNA antibodies and their relationship to the response to foreign antigens. We therefore immunized young NZBWF1 mice with PC-KLH and showed that their immune response was dominated by T15 (S107) antibodies that use the same genetic elements (Vn1, DFL-16, Jn1, Vn22, and Jn5) as the nonautoimmune BALB/c mice (reference 15 and Table 1). When such PC-immunized NZBWF1 mice had developed IgG anti-dsDNA antibodies, they were boosted with PC-KLH and their spleen cells were fused 3–4 d later. The fusions from two animals (N4 and N14) were screened by RNA dot blot (18) and 19 hybrids (Groups 1–7) expressing members of the Vn S107 family were sequenced and examined for their ability to bind various antigens. Based on the sequence of the four members of the Vn S107 germline genes from both NZB and NZW mice (14, 15, and our unpublished results), we were able to determine that all 19 of these antibodies were derived from Vn11 germline genes (Table 1 and Fig. 2).

Antigen Binding. The 19 Vn11-encoded mAbs were examined for their ability to bind dsDNA and ssDNA using a solid phase ELISA. Four patterns of DNA binding were observed and are illustrated in Fig. 1 and summarized in Table 1, where the antibodies are grouped into families that utilize the same genetic elements and share the same junctional and N sequences (see below). In Fig. 1, N4-18 is an IgG2a antibody from a family of nine antibodies (Table 1, group 2), all of which bind dsDNA more strongly than ssDNA. Since anti-DNA binding can be detected with <2 μg of N4-18, its binding is comparable with that of the anti-dsDNA antibodies that have been reported by others (41, 42).

N4-3 (Table 1, group 4) reacts with ssDNA and weakly, if at all, with dsDNA (Fig. 1). N4-4 is an IgG2a antibody from the second (N14) mouse (Table 1, group 1) and it binds more strongly to ssDNA than to dsDNA. Even though N4-26 is also an IgG2a antibody that is encoded by the Vn11 gene, it does not bind dsDNA or ssDNA (Fig. 1).

Genetic Elements. By sequencing the heavy chain V regions of the 19 antibodies in groups 1–7 (Table 1), we discovered that they contain base changes that distinguish the Vn11 germline gene from the Vn1, Vn3, and Vn13 members of the S107 family in both NZB and NZW mice (43). The antibodies could be divided into groups based on their utiliza-
The specificity and variable region gene usage of the MZBWFI hybridomas. Group 1 hybridomas are from the N14 fusion, group 2-7 hybridomas are from the N4 fusion, and group 8 hybridomas are from the N8 fusion. Hybridoma supernatants are tested for reactivity with PC and DNA. The assignment of heavy chain variable region gene usage is based on the sequences of the relevant NZB and NZW germline genes. The D and J elements are based on sequence homology to BALB/c elements. The VK subgroups are based on the classification of Kabat et al. (46) and for the subgroups listed, are identical to the group numbers assigned by Potter (61). The D, JH, and Jr segments were compared to BALB/c germline sequences (46). D42 and 6G6 are two V\(_{11}\)-encoded antibodies taken from the literature (11,36); their specificity is also shown.

| Group | Antibody | PC | DNA | Class | VH | D | JH | Average R/S VH | VK | JK |
|-------|----------|----|-----|-------|----|---|----|-------------|----|----|
| 1     | N14-4,6,8 | +/-| ss>ds| IgG2a | 11 | FL16.1 | 3 | 4.3/0.3 | VK8 | 2 |
| 2     | N4 (9)   | -  | ds>ss| IgG2a | 11 | Q52  | 1 | 6.6/4.2 | VK4,5 | 5 |
| 3     | N4-23,38 | -  | ds = ss| IgG2a | 11 | SP2.2 | 2 | 2.0/2.5 | VK4,5 | 4 |
| 4     | N4-3,6   | -  | ss  | IgG3  | 11 | SP2.3 | 2 | 3/0      | ?   | 3 |
| 5     | N4-24    | +  | -   | IgM   | 11 | SP2.3 | 2 | 1/0      | VK19 | 5 |
| 6     | N4-26    | -  | -   | IgG2a | 11 | Q52  | 2 | 0        | VK4,5 | 2 |
| 7     | N4-31    | -  | -   | IgM   | 11 | Q52  | 2 | 0        | VK8  | 4 |
| 8     | N8-1,5,24| +  | -   | IgM   | 1   | FL16.1 | 1 | -        | VK22 | 5 |
| 9     | 6G6      | +  | +   | IgM   | 11 | Q52  | 3 | 5/11     | ?   | 5 |
| 10    | D42      | -  | +   | IgG2a | 11 | SP2.3 | 1 | 3/0      | ?   | 5 |

The specificity and variable region gene usage of the MZBWFI hybridomas. Group 1 hybridomas are from the N14 fusion, group 2-7 hybridomas are from the N4 fusion, and group 8 hybridomas are from the N8 fusion. Hybridoma supernatants are tested for reactivity with PC and DNA. The assignment of heavy chain variable region gene usage is based on the sequences of the relevant NZB and NZW germline genes. The D and J elements are based on sequence homology to BALB/c elements. The VK subgroups are based on the classification of Kabat et al. (46) and for the subgroups listed, are identical to the group numbers assigned by Potter (61). The D, JH, and Jr segments were compared to BALB/c germline sequences (46). D42 and 6G6 are two V\(_{11}\)-encoded antibodies taken from the literature (11,36); their specificity is also shown.

![Figure 1](image_url) Figure 1. The binding of representative antibodies to dsDNA and ssDNA. Direct binding was measured by ELISA using culture supernatants. The supernatants were all adjusted to 50 µg/ml and then serially diluted. All four antibodies are IgG2a and encoded by the V\(_{11}\) heavy chain gene.

Table 1. The Specificity and Variable Region Gene Usage of the NZBWFI Hybridomas

The specificity and variable region gene usage of the NZBWFI hybridomas. Group 1 hybridomas are from the N14 fusion, group 2-7 hybridomas are from the N4 fusion, and group 8 hybridomas are from the N8 fusion. Hybridoma supernatants are tested for reactivity with PC and DNA. The assignment of heavy chain variable region gene usage is based on the sequences of the relevant NZB and NZW germline genes. The D and J elements are based on sequence homology to BALB/c elements. The VK subgroups are based on the classification of Kabat et al. (46) and for the subgroups listed, are identical to the group numbers assigned by Potter (61). The D, JH, and Jr segments were compared to BALB/c germline sequences (46). D42 and 6G6 are two V\(_{11}\)-encoded antibodies taken from the literature (11,36); their specificity is also shown.
Somatic Mutation of Anti-DNA Antibodies
tion of two V region amino acids at the VJ junction and does not associate with the SI07 heavy chain and is therefore not secreted (29). The group 2 and 3 light chains are members of the V14 or V15 families and are very similar to each other and to SI07B in BALB/c (Fig. 3). The light chain used by the three N14 anti-DNA antibodies (group 1) belongs to the V18 subgroup and closely resembles the McPC 603 light chain, which is found in many PC binding antibodies, especially those made in response to immunization with Proteus morgani (47). These light chains are most homologous to V1G15, which in combination with J12, forms the light chain used by HPCG15 (38), a BALB/c anti-PC mAb encoded by Vn11. This is particularly interesting in light of the ability of the group 1 antibodies to bind PC, albeit weakly. A very similar light chain is present in the IgM (group 7) antibody from the N4 fusion. The V1G19 like light chain that is found in N4-24, a Vn11-encoded anti-PC antibody (group 5), has to our knowledge not previously been associated with the anti-PC response. However, it does have the Tyr at residue 94, Pro at residue 95, and Leu at residue 96, which are thought to be important for PC binding (48).

Somatic Mutations. The N14 (group 1) hybridomas bind ssDNA better than dsDNA (Fig. 1), react with Crithidia, and also bind weakly to PC, cardiolipin, the influenza hemagglutinin, and protamine (data not shown). They each have four to six replacement substitutions in their heavy chain V regions (Table 2) with an average frequency of 2.4% in the CDRs and 1.0% in their framework (FW) regions. They have a replacement (R) to silent (S) ratio in both their CDRs and FWs that is >1.5:1, which is found in most FWs (Table 2) (44). Based on these criteria (35, 44, 45), the three N14 antibodies represent the progeny of single B cell that have undergone a moderate amount of somatic mutation and could have been driven to proliferate and selected for amplification by antigen.

Using similar criteria, N4-23 and 38 (Table 1, group 3), which bind dsDNA and ssDNA, also appear to be derived from a single parental B cell (Figs. 2 and 3). They contain a few somatic mutations but do not have a high R/S ratio (Table 2). N4-3 and 6 are IgG3 antibodies that bind ssDNA and are also related to each other by shared junctional sequences, light chains, and a few common and unique replacements (Table 1, Figs. 2 and 3). The C-A substitution in J2, which is used by N4-23, 38, 3, 6, 24, 26, and 31, is probably a polymorphism since it is found in all NZBWF1 H chains that use J2.

The nine group 2 antibodies are the largest and most interesting family from the N4 fusion. Their isotype is IgG2a (Table 1) and several are cationic with isoelectric points between 7.2 and 8.0 (data not shown). They vary greatly in their binding to dsDNA (Fig. 4) and do not bind PC or cardiolipin. All nine antibodies appear to be the progeny of a single B cell based on (a) utilization of Vn11, DQ32, and Jn1 genetic elements to form the heavy chain V region; (b) the same apparent N sequences and a junctional recombination site that converts the first amino acid of Jn1 from a tyrosine (TAC) to an asparagine (AAC); and (c) the use of the same Vn germine gene and Jn5 (Table 1 and Figs. 2 and 3). In addition, many of these hybridomas share the same novel nonproductive rearrangements and those that lack these fragments have presumably lost them due to segregation after fusion (data not shown).

All members of this family have many somatic mutations in both the heavy and light chain genes (Fig. 2–4). While there are some shared mutations among subsets of two or three antibodies, it is not possible to organize them in a manner in which more than two form a branch of a family tree and most seem to represent distinct sublineages of the parental B cell. For example, N4-16, 10, 36, and 27 share a G-A substitution that encodes a glycine (GGT) instead of the germ-line encoded aspartic acid (GAT) at residue 54 in CDR2. However, each of these antibodies has distinctive mutations shared with other members of group 2, making it difficult to determine if they are on the same branch of the family tree or if there are many parallel mutations (49). Similarly, N4-18, 10, and 1 all share a G-T change in the second residue of J and N4-10 and 1 both have a G-A change in that same codon (Fig. 2). If the G-T in N4-18 occurred first, then it would be the parent of N4-10 and 1. However, N4-18 contains many base changes that are not present in N4-1 or 10. It is possible that the parent of N4-18 was also the parent of N4-1 and 10 but this is difficult to prove. Parenthetically, if the G-A had occurred first in N4-10 and 1, it would have created a nonsense mutation (TAG) but the G-T substitution protects from that.

Table 2. R/S Ratios in Heavy Chains

| Group | Antibody | CDR | FW | Total |
|-------|----------|-----|----|-------|
| 1     | N14-4    | 2/0 | 2/0| 4/0 4 |
|       | N14-6    | 2/0 | 2/1| 4/1 5 |
|       | N14-8    | 3/0 | 4/0| 7/0 7 |
| 3     | N4-23    | 1/1 | 1/1| 2/2 4 |
|       | N4-38    | 1/2 | 1/1| 2/3 5 |
| 4     | N4-3     | 3/0 | 0/0| 3/0 3 |
|       | N4-6     | 1/0 | 0/0| 1/0 1 |

R/S ratios in the heavy chains of groups 1, 3, and 4. The ratio of mutations that result in an amino acid replacement (R) to those that are silent (S) mutations are tabulated for the FWs and the CDRs as defined by Kabat et al. (46).
Somatic Mutation of Anti-DNA Antibodies
We also examined the switch sites of N4 hybridomas to identify members of a single branch of the family tree. For example, only N4-36 and 17 have identical switch recombination sites when probed with a γ2k switch site probe (data not shown). They also show a shared single silent mutation at residue 58 but have no other shared mutations. This suggests that they were derived from a common precursor which switched after acquiring the shared mutation at residue 58 and then underwent subsequent divisions and acquired a number of independent mutations all following switching. Similar observations have been reported by others (50, 51) and suggest that point mutations continue to arise following switching from IgM to IgG.

Since we were unable to organize this group of nine antibodies into a genealogy that contained more than two to three members per branch, we attempted to examine their relationship to each other by comparing their binding to dsDNA (Fig. 4). N4-16, 18, 10, and 36 show easily detectable binding to dsDNA at 1-2 μg/ml. However, they do not have many more V region mutations than N4-17 and 19, which bind weakly. It is worth noting that N4-17 and 19 would probably not have been identified if we had used antigen binding to screen the hybridomas since even at 10 μg/ml, which is a reasonably high concentration of antibody for an average hybridoma, their DNA binding is barely above the background. N4-1, 2, and 27 have the least number of somatic mutations and require 5-10 μg/ml to give detectable binding. Thus, the number of V\(\alpha\) mutations is not directly proportional to the binding of dsDNA.

It was possible that N4-17 and 19 had initially acquired higher binding due to somatic mutation and that the process of maturation continued and resulted in a decrease in their binding. One might expect that this would be reflected in the location of the replacement mutations in these antibodies. However, N4-17 does not have any replacement substitution in its heavy chain CDRs and the replacement substitutions in the CDRs of N4-19 are not shared with any of the highest binders suggesting that these are antibodies that never underwent somatic mutation that would increase their binding to dsDNA or a cross reactive antigen. The heavy chain CDRs of N4-10 and N4-1 have R to S mutations that exceed the 2.9:1 ratio which is thought to occur randomly (52), but many of the other antibodies of this group have R/S ratios in their heavy chains CDRs, which are less than the 1.5:1 ratio found in the FW residues of antibodies in general (Fig. 4) (44).

The NZW germline VK gene, which encodes the light chains of these nine antibodies, was cloned by taking advantage of its homology with the S107B VK gene segment (29). The relevant NZB and NZW genes did not differ by restriction analysis and hybridization with various oligonucleotides including one that spans the deletion (data not shown). The sequence of the NZW VK gene (Fig. 3) is identical to the consensus sequence that was derived from all nine light chains (14). The most remarkable finding is that N4-16 and 18 have very high R/S ratios in the FWs of their light chains (Fig. 4). These FW changes in the light chains of N4-16 and 18 are distributed throughout the linear sequences of these antibodies (Fig. 3). It is interesting to note that there are many replacement substitutions in the FW of all of the light chains except N4-10.

Since we screened for the expression of V\(\alpha\)S107 by RNA dot blot, we also identified antibodies from the N4 animal that did not bind DNA (Table 1). The nonDNA binders use the same genetic elements as the DNA binders but in different combinations (Table 1). N4-24 utilizes the V\(\alpha\)11 gene, does not bind DNA, but does bind PC. Other V\(\alpha\)11-encoded PC-
binding antibodies have been reported. HPCG15, a BALB/c
V_{\mu}11-encoded anti-PC antibody has undergone extensive so- 
matic mutation (38), while V_{\mu}11 anti-PC antibodies from 
other mouse strains, such as CBA/J (53) and CLA-2/Cn (37), 
are germline encoded.

N4-24 shows that the same heavy chain variable region 
gene can encode antibodies that react with DNA and with 
a foreign antigen at the same time in a single mouse. N4-26 
does not bind DNA or PC and does not have any somatic 
mutations in the V_{\mu}11 gene (Fig. 2). N4-31 has a single con-
servative (Val to Ile) amino acid substitution in the third FW.

Discussion

We have determined the variable region sequences of 19 
antibodies expressing the S107 V_{\mu}11 germline from two 
NZBWF1 mice. 16 of these antibodies bind DNA and are 
the products of four different B cell clones. Based on serum 
studies and screening of a number of fusions, we know that 
V_{\mu}11-encoded antibodies represent ~5–30% of the anti-
dsDNA antibodies made by older autoimmune NZBWF1 
mice (reference 15 and our unpublished studies). However, 
by focusing our attention on V_{\mu}11-encoded antibodies, we 
were able to determine the sequences of the relevant germ-
line genes and to screen hybridomas for V_{\mu} expression in 
order to recover antibodies that might have been missed if 
we had only looked for those that bound antigen. Although 
the animals were immunized with PC, and many of the anti-
bodies used genetic elements associated with the anti-PC re-
response, further experiments will have to be done to deter-
mine the influence of the prior immunization of these animals 
with PC-KLH and the boost with this antigen immediately 
preceding the fusions.

All of the anti-DNA antibodies that we have identified 
have undergone significant somatic mutation. It is not clear 
whether somatic mutation is required to create pathogenic 
autoantibodies. For example, Naparsteck et al. (54) have shown 
that a germline-encoded antibody from a nonautoimmune 
mouse combined with both a foreign antigen and DNA, and 
that the progeny of this antibody lost their DNA binding 
as they underwent somatic mutation and affinity maturation 
for the foreign antigen. No V_{\mu}11 germline-encoded DNA 
binding antibodies were identified in the fusions that we have 
carried out. This suggests that either the germline V_{\mu}11 
cannot achieve sufficient affinity for dsDNA irrespective of 
the N and D sequences with which it is associated or that 
B cells expressing high affinity germline-encoded V_{\mu}11 au-
toantibodies are suppressed. The latter is more likely since 
Shafner et al. (55) have shown that V_{\mu}11 germline-encoded 
antibodies with strong DNA binding can be detected in 
BALB/c mice whose suppressive mechanism has been blocked.

The presence of replacement substitution in the CDRs and 
the ratio of R to S mutations can be used to deduce whether 
a particular antibody response is driven by antigen and whether 
the antigen selectively promotes the outgrowth of B cells 
making higher affinity antibodies (35, 44, 45). Some of the 
antibodies that we have identified have high R/S ratios in 
their CDRs suggesting that the amino acid substitutions in 
their antigen binding site has increased their ability to bind 
antigen. The analysis of the nine antibodies in group 2 sug-
gests that some of the B cells in this clone are the product 
of such an antigen-driven and selected response and that the 
antigen is either dsDNA or crossreactive with it, i.e., the 
members of this group with the lowest number of replace-
ment substitutions in their CDRs bind DNA poorly and 
the highest binders have a number of replacement substitu-
tions in their binding sites. This suggests that, at least for 
this B cell clone, somatic mutation is required to achieve 
significant binding to DNA. In addition, these antibodies 
have some of the characteristics that have been suggested by 
others (34, 42, 56, 57) to promote binding to DNA. For 
example, there are arginines and asparagines in the third CDR 
of this group. N4-18, which is one of the highest binding 
members of the group, has acquired an additional arginine 
in that region through somatic mutation. However, at least 
in the assays that we have used, the binding of N4-18 to 
dsDNA is no better than that of N4-16 (Fig. 4), which does 
not have that particular somatic mutation. Eilat et al. (34) 
and Shlomchik et al. (56) have noted that some anti-DNA 
antibodies express their D regions in unusual reading frames, 
have inverted Ds, or are the product of D-D recombination.
These characteristics are not present in the V_{\mu}11-encoded anti-
bodies that we have studied, confirming that some anti-
DNA antibodies can acquire strong binding without unusual 
forms of D (56).

The presence of somatic mutations and the IgG2a isotype 
indicate that helper T cells have interacted with B cells in 
order to generate the anti-DNA antibodies that we have 
studied. It is more difficult to deduce the role of antigen. 
However, it is worth noting that at least in one instance, 
somatic mutation has led to a B cell clone that started out 
producing antibodies to only ssDNA to produce an antibody 
that also reacted with dsDNA (56), again suggesting a role 
for dsDNA, or something highly crossreactive with it, in 
driving the anti-DNA response. While the antibodies in group 
1 and group 4, and some of the antibodies in group 2, have 
high R/S ratios in their CDRs, most of the antibodies in 
group 2 and group 3 do not have these characteristics. This 
is also true of some of the anti-DNA antibodies that have 
been described by others (56).

Since we screened our fusions for V region utilization rather 
than antigen binding, it is difficult to compare our results 
with most of the studies in the literature where the anti-
bodies have been identified by their ability to bind antigen. 
Nevertheless, genealogies resembling the group 2 antibodies 
with their many independent sublineages have been observed 
in responses to haptens (50, 59). Manser (50), who also 
screened fusions for V region utilization, reported a large family 
of antibodies that were made in response to a foreign antigen 
in which there were many R substitutions in the FWS of 
the light chains. That particular clone is unusual, in that it 
may have lost the expression of its surface Ig due to a non-
sense mutation early in its propagation, undergone a period 
of unselected mutations, then reverted to the nonsense muta-
tion and gone on to expand the clone under the influence of 
antigen (50).
In spite of these precedents in other studies, it is unusual to find a single clone that contains antibodies like N4-17 and N4-19, which bind weakly to antigen (59), that has progeny with many independent sublineages, and whose antibodies have so many R substitutions in the FW of the light chains. It is possible that some of these changes in the FW of the light chains have a direct effect upon the conformation of the antigen-binding site. However, it is also possible that antidiotype antibodies or idiotype-specific T cells (60) contributed to the expansion of the B cell producing the group 2 antibodies and perhaps some of the other antibodies we have identified. Further studies will be required to determine whether the peculiarities of the group 2 antibodies are a reflection of abnormalities that result in the production of autoantibodies by autoimmune mice or whether similar responses will also be found in the response of nonautoimmune mice to foreign antigens.

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