Characterization of Somatically Mutated S107 V_H11-encoded Anti-DNA Autoantibodies Derived from Autoimmune (NZB × NZW)F_1 Mice

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Summary

We have studied 19 S107 heavy chain variable region gene (V_H11)-encoded monoclonal antibodies from NZBWF1 mice. These studies show that a single V_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)[1] (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)F_1 (NZBWF1) (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 nonpathogenic. 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_HS107 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_HS107 family from an autoimmune NZBWF1 mouse and showed that they were encoded by the V_H11 member of the V_HS107 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_H11-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.

[1] *Abbreviations used in this paper:* dsDNA, double stranded DNA; FW, framework; (NZB × NZW)F_1, NZBWF1; PC, phosphorylcholine; R, replacement; S, silent; ss, single stranded DNA; V_H, heavy chain variable region gene.

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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_HS107 family were detected by RNA dot blot hybridization (18) and then cloned twice in soft agar before characterization (19).

The different V_HS107 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. Afterwards, the solution was flicked out and the plates rinsed with distilled water. DNA was diluted in 1× SSC and plates were coated with 100 μl/well and incubated overnight at 4°C. The next day the plates were washed three times with PBS-tween 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 lucilae* (24) using a commercially (Clinical Sciences, Whippany, 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 V_H 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_K 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_K light chain consensus sequence of the group 2 (Table 1) antibodies from the BALB/c V_K S107B germline and the group 3 V_K 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-[^{35}S] thiol triphosphate and M13 vectors (31).

Results

The conversion of S107 from a protective antibody to an autoantibody indicated that the V_H1 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_H1 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 V_H11 genes showed that they encode a serine at residue 35 and that V_H11 was the germline gene for the one S107-like NZBWF1 antibody that had been reported (11, 34). Thus the germ line V_H11 is similar to the DNA binding mutant of V_H1 and can encode DNA binding proteins. The V_H11 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 V_HS107 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 (V_H1, DFL-16.1, J_H1, V_K22, and J_K5) 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 V_HS107 family were sequenced and examined for their ability to bind various antigens. Based on the sequence of the four members of the V_HS107 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 V_H11 germline genes (Table 1 and Fig. 2).

Antigen Binding. The 19 V_H11-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). N14-4 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 V_H11 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 V_H11 germline gene from the V_H1, V_H3, and V_H13 members of the S107 family in both NZB and NZW mice (43). The antibodies could be divided into groups based on their utiliza-

Table 1. *The Specificity and Variable Region Gene Usage of the NZBWF1 Hybridomas*

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		511	?
10	D42	−	+	IgG2a	11	SP2.3	1	3/0	?	5

The specificity and variable region gene usage of the MZBWF1 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 V_κ 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, J_H, and J_K segments were compared to BALB/c germline sequences (46). D42 and 6G6 are two V_H11-encoded antibodies taken from the literature (11,36); their specificity is also shown.

tion of the various germline elements that form the mature V regions (Table 1). Furthermore, examination of the junctional areas in the third CDR of the heavy chain V regions, including potential N regions, and of shared somatic mutations in the V regions, indicates that each of the groups in Table 1 represent the progeny of a single B cell (35, 44, 45). For example, the heavy chains of all three antibodies from the N14 fusion (Table 1, group 1) use the FL16.1 D region and J_H3, differ from the germline V_H11 region by replacement substitutions at residues 31, 44, 52A, and 93 (Fig. 2), and have a nongermline-encoded junctional arginine in CDR3 of the heavy gene. However, they each differ from the others by one or two base changes (Fig. 2). Their light chains are encoded by a V_K8-like gene and J_K2 and differ from the V_KG15 sequence (kindly provided by Dr. P.J. Gearhart, Johns Hopkins Medical School) by six to seven bases (Fig. 3 and Table 1). The V_K differences shared by the three light chains could be polymorphisms since we do not know the germline sequence of the relevant V_K gene in NZB or NZW mice. When the DNA from these three hybridomas was examined by Southern analysis with a J_H region probe, all shared novel restriction fragments that are not present in the liver of NZB or NZW mice or in the NSO fusion partner (data not shown). Taken together, these findings suggest that these three antibodies are all derived from a single B cell clone (35, 44, 45).

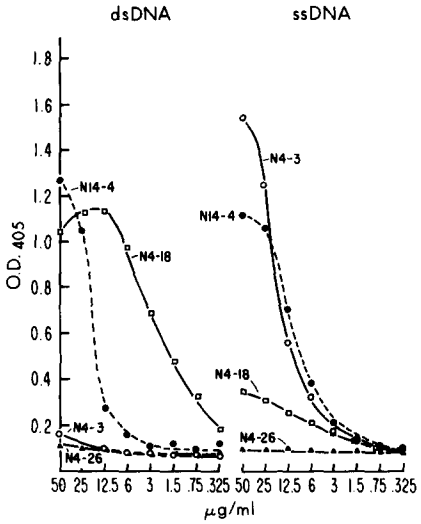
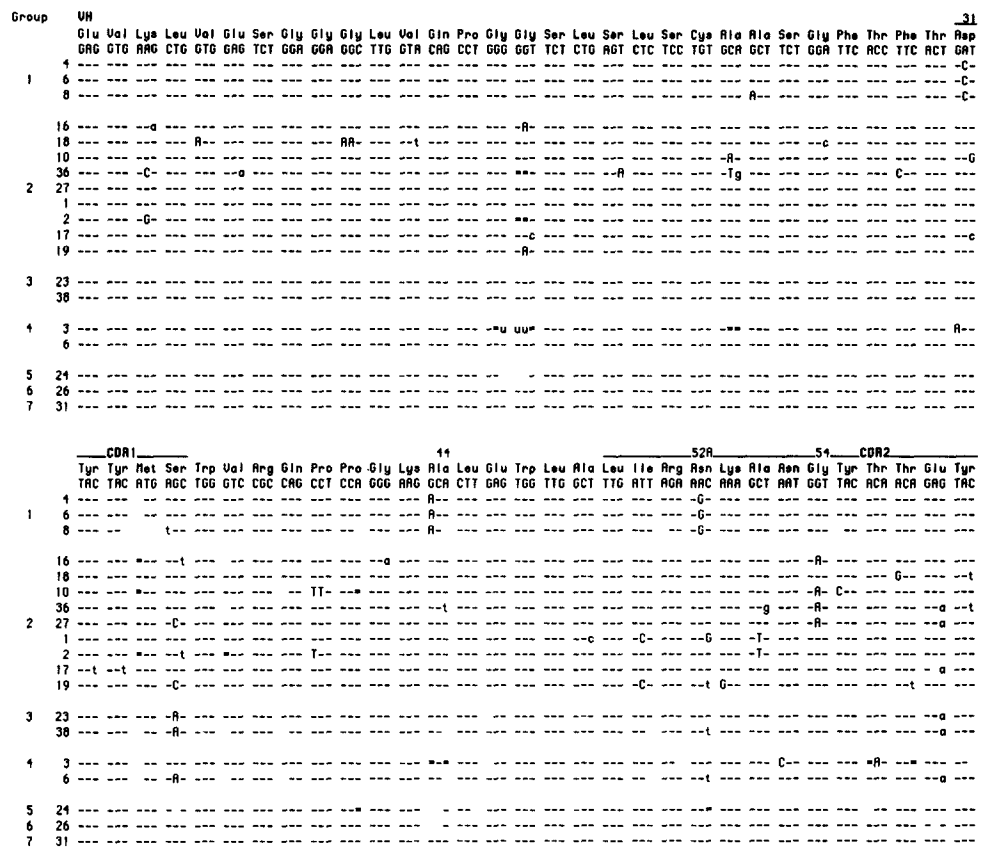


Figure 1. The binding of representative antibodies to dsDNA and ssDNA. Direct binding was measured by ELISA using culture supernatants. The supernatants were all adjusted to 50 μg/ml and then serially diluted. All four antibodies are IgG2a and encoded by the V_H11 heavy chain gene.

Similar analysis of the rest of the antibodies made it possible to identify the other genetic elements that are associated with V_H11 in the N4 animals. As shown in Table 1, four different D and three different J segments were found associated with V_H11 in the DNA binding antibodies that were identified. Three of the four Ds were also found in the PC binding and non-DNA non-PC binding antibodies from the N4 animal. The fourth D region, DFL16.1, was present in all of the PC binding antibodies encoded by the V_H1 germline gene from a younger animal (Table 1, group 8) (15). The light chains that were found in these antibodies were also somewhat restricted in that they all had some association with the S107 family or the PC response. The light chains expressed in groups 2 and 3 (Table 1), which bind dsDNA, and in the non-DNA binding IgG2a (group 6), were V_k genes that closely resemble S107B. S107B is a light chain expressed in large amounts in the S107 tumor and cell line that has a dele-



tion of two V region amino acids at the VJ junction and does not associate with the S107 heavy chain and is therefore not secreted (29). The group 2 and 3 light chains are members of the V_k4 or V_k5 families and are very similar to each other and to S107B in BALB/c (Fig. 3). The light chain used by the three N14 anti-DNA antibodies (group 1) belongs to the V_k8 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 V_kG15, which in combination with J_k2, forms the light chain used by HPCG15 (38), a BALB/c anti-PC mAb encoded by V_H11. 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 V_k19 like light chain that is found in N4-24, a V_H11-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 J_H2, 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 J_H2.

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 V_H11, D_{Q52}, and J_H1 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 J_H1 from a tyrosine (TAC) to an asparagine (AAC); and (*c*) the use of the same V_K germline gene and J_K5 (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).

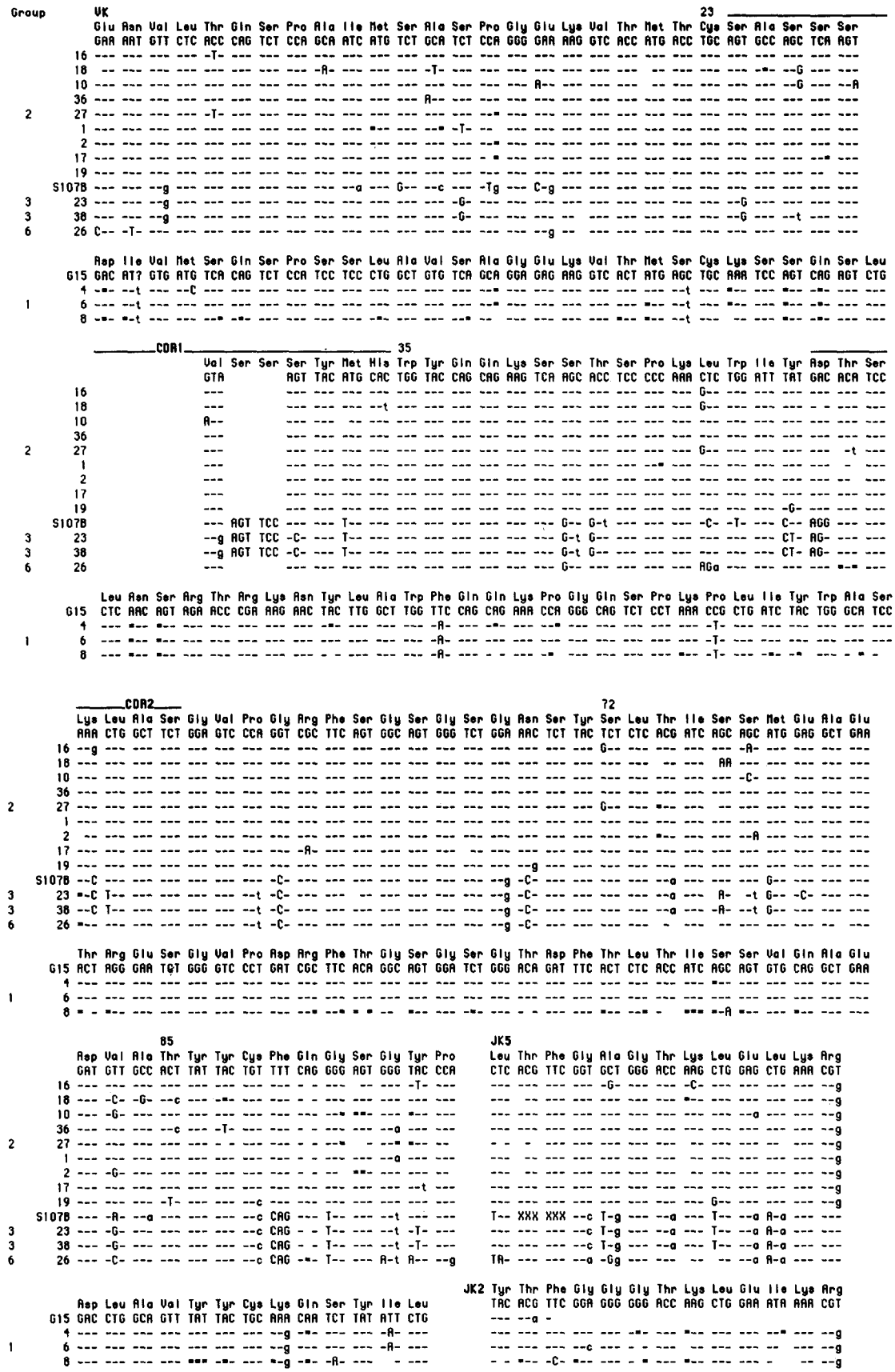
All members of this family have many somatic mutations in both the heavy and light chain genes (Fig. 2–4). While there are some shared mutations among subsets of two or three antibodies, it is not possible to organize them in a manner in which more than two form a branch of a family tree and most seem 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 germline 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).

Figure 2. Heavy chain variable region sequences of NZBWF1 V_H11-encoded antibodies. The heavy chain variable region sequences for the 19 antibodies from the N4 and N14 fusions are shown compared with the NZB and NZW V_H11 germline sequences. The NZB and NZW V_H11 are identical to each other (14, 43). The N and D sequences from the hybridomas are compared with the consensus sequences derived from each group. The BALB/c germline J_H1, J_H2, and J_H3 sequences are shown for comparison. Numbering is according to Kabat et al. (46). The symbols are as follows: (–) identity; (=) probable identity; (u) purine; (y) pyrimidine; (lower case letter) silent mutation; (upper case letter) replacement mutation; (space) ambiguous base.



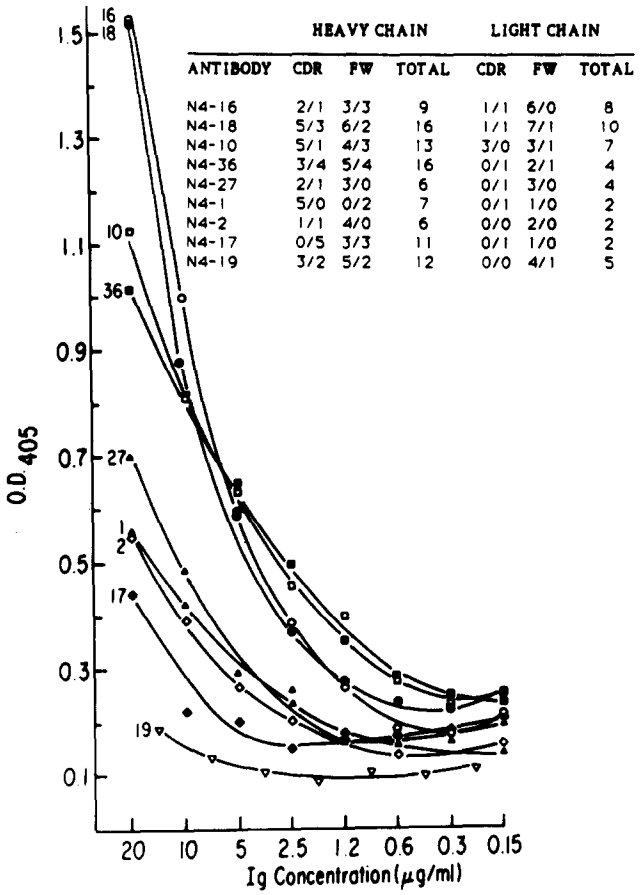


Figure 4. Direct binding to dsDNA. The nine group 2 V_H11-encoded anti-dsDNA antibodies were tested for dsDNA binding by a direct ELISA. The culture supernatants were adjusted to 20 μg/ml and then serially diluted.

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 γ_{2a} 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_H 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 V_k gene, which encodes the light chains of these nine antibodies, was cloned by taking advantage of its homology with the S107B V_K 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 V_K 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_HS107 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_H11 gene, does not bind DNA, but does bind PC. Other V_H11-encoded PC-

Figure 3. Light chain variable region sequences of V_H11 anti-DNA antibodies. The light chain variable region sequences for 14 anti-dsDNA antibodies from the N4 and N14 fusions are shown compared with the NZW-cloned V_K gene (*top line*), as well as the BALB/c S107 and V_KG15 light chains (nucleotide sequence of V_KG15 kindly provided by Dr. P.J. Gearhart). The J_K segment is compared with the BALB/c germline J_K2 and J_K5 sequence. The symbols are as follows: (x) deleted base; (–) identity; (=) probable identity; (lower case letter) silent mutation; (upper case letter) replacement mutation; (space) ambiguous base.

binding antibodies have been reported. HPCG15, a BALB/c V_H11-encoded anti-PC antibody has undergone extensive somatic mutation (38), while V_H11 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_H11 gene (Fig. 2). N4-31 has a single conservative (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_H11 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_H11-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_H11-encoded antibodies, we were able to determine the sequences of the relevant germline genes and to screen hybridomas for V_H 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 antibodies used genetic elements associated with the anti-PC response, further experiments will have to be done to determine 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, Naparstek 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_H11 germline-encoded DNA binding antibodies were identified in the fusions that we have carried out. This suggests that either the germline V_H11 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_H11 autoantibodies are suppressed. The latter is more likely since Shafner et al. (55) have shown that V_H11 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 suggests 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 replacement substitutions in their CDRs bind DNA poorly and the highest binders have a number of replacement substitutions 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_H11-encoded antibodies 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 antibodies 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 nonsense mutation early in its propagation, undergone a period of unselected mutations, then reverted to the nonsense mutation 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 antiidiotypic 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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