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Primary Structures and Chain Dominance of Anti-DNA Antibodies

Jeong Soo Park[1], Young-Tai Kim[C], Hee Yong Chung[2], Kwanghee Baek[1], and Young-Ju Jang*

Laboratory of Immunology, Institute for Medical Science, Ajou University School of Medicine, Suwon 442-749, Korea;
[1]Department of Genetic Engineering, Kyung Hee University, Suwon 449-701, Korea;
[2]Department of Microbiology, Hanyang University College of Medicine, Seoul 133-791, Korea.

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Using several anti-DNA autoantibodies, we analyzed the relative involvement of heavy and light chains in their interactions with DNA. We previously obtained eight hybridomas producing monoclonal anti-DNA autoantibodies by fusing spleen cells from an MRL-*lpr/lpr* mouse with myeloma cells. The chain dominance was analyzed by UV cross-linking experiments, in which the antibodies were covalently cross-linked with radioisotope-labeled oligonucleotides by short-wavelength UV-light, and the cross-linked H and L chains were analyzed by SDS-PAGE and densitometric scanning. Among these, three were found to be heavy chain dominant antibodies in which heavy chains are dominantly involved in DNA binding. The other five were co-dominant antibodies in which both heavy and light chains are involved in DNA binding. To determine the factor(s) that can explain the chain dominance in DNA binding, we determined the amino acid sequences of the variable regions of both heavy (VH) and light (VL) chains of all eight monoclonal antibodies. By analyzing the data, we were able to draw the following conclusions: (1) The arginine residues are found in the CDR3 regions of both VH and VL of the co-dominant antibodies; whereas, the same residues are found only in the CDR3s of VH, but not in VL, of the heavy chain dominant antibodies. (2) The net charges of the V regions affect the chain dominance. From the results of this study it is suggested that the presence of arginine residue in CDR3 is a critical factor in determining chain-dominance, as well as DNA binding of anti-DNA antibodies in general.

Keyword: Anti-DNA Antibody; Arginine; Chain Dominance; Net Charge; VH; VL.

[C] Present address: P.O.Box 42, Granville, N.Y. 12832, U.S.A.

* To whom correspondence should be addressed.
Tel: 82-31-219-4516; Fax: 82-31-219-4503
E-mail: jangyj@madang.ajou.ac.kr

Introduction

Systemic Lupus Erythematosus (SLE) is a multisystemic autoimmune disease caused by immune response to self antigen. Studies on the biochemical properties of anti-dsDNA antibodies have been an interesting area, because their specificity for dsDNA is correlated to their pathogenicity, and their concentrations are associated with active disease status (Koffler *et al.*, 1967; Krishnan *et al.*, 1996; Stollar, 1981; Tan, 1991).

In general, both heavy (H) and light (L) chains of antibodies appear to contribute to the binding to antigens. However, several studies with anti-DNA antibodies demonstrated that the cooperation between the H and L chains is not always necessary for antigen binding. We previously reported that dsDNA was cross-linked only to the H chain of 2C10, an anti-DNA monoclonal autoantibody derived from an MRL-*lpr/lpr* mouse, in UV cross-linking experiments (Jang *et al.*, 1990). The H chain dominance in the binding of DNA by 2C10 was further confirmed by site directed mutagenesis experiments using scFv (Jang *et al.*, 1996; 1998). Other examples of H chain dominant antibodies include Z22, a monoclonal anti-Z-DNA antibody from immunized C57BL/6 mice, and 3H9, a monoclonal anti-DNA autoantibody from an MRL-*lpr/lpr* mouse (Polymenis and Stollar, 1995; Radic *et al.*, 1991). On the other hand, there were examples in which both the H and L chains were involved in the interaction with DNA. We previously showed that both the H and L chains of a monoclonal anti-dsDNA autoantibody from an MRL-*lpr/lpr*, H241, were involved in the interaction with DNA (Jang *et al.*, 1990). A crystallographic analysis of a mouse anti-ssDNA autoantibody, BV0401, with bound $(dT)_3$, also revealed the importance of amino acids from both chains (Herron *et al.*, 1991; Rumbly *et al.*, 1993).

As the chain dominance was analyzed only in several anti-DNA antibodies derived from different strains of mice, we decided to analyze the phenomenon more systematically by generating a number of anti-DNA

monoclonal antibodies from an *MRL-lpr/lpr* mouse and analyze them in terms of chain-dominance and primary amino acid sequences. Our results show that the H chain dominance is not a rare phenomenon among the anti-DNA antibodies. Also, the presence of arginine in CDR3 and net charges of V regions determine whether or not a particular chain would make direct contact with DNA.

Materials and Methods

Production of monoclonal antibodies Monoclonal anti-DNA antibodies were obtained by fusing spleen cells from a 6 month-old MRL-*lpr/lpr* mouse with SP2/0 myeloma cells in the presence of 50% polyethylene glycol (PEG) 4000 (mol. wt. 3,000−4,000) (GIBCO BRL, USA) as described earlier (Galfre *et al.*, 1977). Hybrid cells were selected in a RPMI medium (Song, 1996) supplemented with Hypoxanthine-Aminopterin-Thymidine (HAT, GIBCO BRL, USA), 20% FBS, and 100 μg/ml penicillin-streptomycin. Cells were grown at 37°C in a humidified incubator supplied with 5% CO_2. Hybridomas secreting anti-DNA antibodies were screened by ELISA with native (double stranded) calf thymus DNA (Sigma, USA) as target antigen (Shoenfeld *et al.*, 1982). Hybridomas screened as positive in anti-DNA antibody secretion were cloned twice more by limiting dilution to establish the clonal populations. The isotypes were determined by using an isotyping kit (Pierce, USA).

Purification of monoclonal antibodies The antibodies were purified by affinity chromatography using an agarose bead coupled to single stranded DNA (Gibco BRL, USA). After passing through the culture supernatant containing the anti-DNA antibodies, the columns were washed with 10 volumes of PBS. The antibodies were eluted from the affinity columns with 700 mM NaCl, and dialyzed in PBS. The concentrations of the antibodies were measured by absorbance at 280 nm.

Direct-binding ELISA Ninety six-well polystyrene microtiter plates (Nunc, Denmark) were incubated with 1 μg/ml calf thymus DNA or with 100 μg/ml yeast RNA (USB, Germany) at 4°C overnight. The plates were washed three times with PBS containing 0.05% Tween 20 (PBST) and blocked with 3% BSA-PBST for 2 h at room temperature (RT). After adding purified monoclonal antibodies, or culture supernatants diluted in PBS, the plates were incubated for 1 h at RT. After washing 3 times with PBST, the amount of antibodies bound to the plates were determined by successive treatment of goat anti-mouse polyvalent Ig conjugated with alkaline phosphatase (Sigma, USA) and p-nitrophenyl phosphate (Sigma, USA). The absorbance was measured at 405 nm using an ELISA reader (Bio-Rad, USA).

Competitive ELISA For competitive assays the monoclonal antibodies were first incubated with various concentrations of polynucleotides: poly(dA)poly(dT), poly(dG)poly(dC), poly(dA-dT) poly(dA-dT), and poly(dG-dC)poly(dG-dC). The concentrations of the monoclonal antibodies used in competitive ELISA were fixed to those can give an O.D. value of 1 at 405 nm in direct-binding ELISA. After incubation, the polynucleotide/ antibody mixtures were transferred to the microtiter plates coated with dsDNA (1 μg/ml). The amount of antibodies bound to the plates was measured by the same method as the direct binding assay.

Band mobility shift assay For band mobility shift assay, the following oligonucleotides were synthesized (Bioneer, Korea) and formed as double strands by boiling at 100°C for 5 min and cooling slowly to RT : oligo-1, d(GAGAGAGAGAGAGAGAGA GA)d(TCTCTCTCTCTCTCTCTC); oligo-2, d(GCGCGATA TATATCGCGC)d(GCGCGATATATATCGCGC); oligo-3, d(A TATAGCGCGCGCTATAT)d(ATATAGCGCGCGCTATAT). These oligonucleotides were radiolabeled at their 5' ends with T4 polynucleotide kinase (Promega, 10 U/μl) and γ-(^{32}P) ATP (Jang and Stollar, 1990). Ten μg of antibody and 250 ng of radiolabeled DNA were mixed and incubated in TBE or TBM buffer for 1 hour at RT. After adding glycerol (30%), the mixtures were separated in native gel electrophoresis and exposed to X-ray film at RT.

UV cross-linking Antibodies (10 μg) were mixed with 250 ng of ^{32}P-labeled oligonucleotides in a total volume of 50 μl, and the mixtures were placed in a polystyrene microtiter plate. The plates were irradiated with UV (280 nm) for 5 min on ice. After UV-irradiation, the mixtures were separated in SDS-PAGE under reducing conditions, and the gel was stained with a 0.1% Coomassie brilliant blue solution for 1 h. The dried gel was exposed to X-ray film at 70°C for autoradiography.

Densitometric scanning The intensity of the individual band on the autoradiogram was measured by two-dimensional densitometric scanning with a Kaiser RSI Image Analyzer and Vilber Lourmat Bio 2D program version 6.32.

DNA sequencing The first strand cDNA was synthesized from the total RNA isolated from hybridoma cells using the universal cDNA synthesis system (Promega, USA) and AccuPowerTM RT PreMix (Bioneer, Korea). The first strand cDNA was amplified by touch-down PCR (94°C 1 min, 65°C 1 min, -0.3°C/cycle; 72°C 1 min, 30 cycles) using leader-sequence primers (MHV primer) and Cγ or Cκ primer as sense and anti-sense primers, respectively (Table 1 and Table 2). MKV or VLFR sense primers were used for the L chains. MKV primers hybridize to the leader sequences of the chain, and the VLFR primer hybridizes to the 5'-end of FR 1 region of the λ chain (Table 2). Primers that hybridize to the constant regions of the κ or λ chains were used as anti-sense primers. Among the primers tested for touch-down PCR, MKV11 (for κ chain), MHV4, 5, or 7 (for H chain), VLFR2 (for λ chain) primers were successful in amplifying the respective V regions. The PCR-products were eluted from the agarose gel and inserted into a pGEM-T easy vector (Promega, USA). DNA sequencing was performed in both directions by an automatic sequencer (ABI Prism 377 DNA Sequencer, Perkin Elmer) using T7 and SP6 promoter-specific primers. At least three cDNA clones obtained from a single hybridoma were subjected to DNA sequencing in order to verify the accuracy of sequencing.

Results and Discussion

Analysis of chain-dominance by UV cross-linking studies To characterize the factor(s) affecting the chain-dominance of antibodies binding to DNA, we generated eight

Table 1. PCR primers for cloning mouse H chain variable regions.

Name of Primers (length of primer)	Sequences[c]
MHV[a]1 (27 mer)	ATGAAATGCAGCTGGGGCATSTTCTTC
MHV2 (26 mer)	ATGGGATGGAGCTRTATCATSYTCTT
MHV3 (27 mer)	ATGAAGWTGTGGTTAAACTGGGTTTTT
MHV4 (25 mer)	ATGRACTTTGGGYTCAGCTTGRTTT
MHV5 (30 mer)	ATGGACTCCAGGCTCAATTTAGTTTAGTTTTCCTT
MHV6 (27 mer)	ATGGCTGTCYTRGSGCTRCTCTTCTGC
MHV7 (26 mer)	ATGGRATGGAGCKGGRTCTTTMTCTT
MHV8 (23 mer)	ATGAGAGTGCTGATTCTTTTGTG
MHV9 (30 mer)	ATGGMTTGGTGTGGAMCTTGCTATTCCTG
MHV10 (27 mer)	ATGGGCAGACTTACATTCTCATTCCTG
MHV11 (28 mer)	ATGGATTTTGGGCTGATTTTTTTTATTG
MHV12 (27 mer)	ATGATGGTGTTAAGTCTTCTGTACCTG
Cγ[b]I	AGGGGCGACTGGATAGAC

[a] MHV indicates primers that hybridize to leader sequences of mouse H chain variable region genes.
[b] Cγ indicates the primer that hybridizes to the mouse H chain constant region gene.
[c] Ambiguity codes: M = A or C; R = A or G; W = A or T; S = C or G; Y = C or T; K = G or T.

Table 2. PCR primers for cloning mouse L chain variable regions.

Name of Primers (length of primer)	Sequences[e]
MKV[a]1 (30 mer)	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTG
MKV2 (30 mer)	ATGGAGWCAGACACACTCCTGYTATGGGTG
MKV3 (30 mer)	ATGAGTGTGCTCACTCAGGTCCTGGSGTTG
MKV4 (33 mer)	ATGAGGRCCCCTGCTCAGWTTYTTGGMWTCTTG
MKV5 (30 mer)	ATGGATTTWCAGGTGCAGATTWTCAGCTTC
MKV6 (27 mer)	ATGAGGTKCYYTGYTSAGYTYCTGRGG
MKV7 (31 mer)	ATGGGCWTCAAGATGGAGTCACAKWYYCWGG
MKV8 (31 mer)	ATCTGGGGAYCTKTTTYCMMTTTTTCAATTG
MKV9 (25 mer)	ATGGTRTCCWCASCTCAGTTCCTTG
MKV10 (27 mer)	ATGTATATATGTTTGTTGTCTATTTCT
MKV11 (28 mer)	ATGGAAGCCCCAGCTCAGCTTCTCTTCC
Cκ[b]I	TGAGGCACCTCCAGATGT
CκII	GGATGGTGGGAAGATGGATAC
CκIII	AGTTGGTGCAGCATCAGC
VLFR[c]2	GAKRCTGTTGTGACTCAG
VLFR3	GACRTYGTGATGACCCAG
Cλ[d]I	CAAACTCTTCTCCACA

[a] MKV indicates primers that hybridize to leader sequences of mouse kappa L chain variable region genes.
[b] Cκ indicates primers that hybridize to the mouse kappa constant region gene.
[c] VLFR indicates primers that hybridize to 5'-end of FR1 of mouse lambda L chain variable region genes.
[d] Cλ indicates primer that hybridizes to the mouse lambda constant region gene.
[e] Ambiguity codes: M = A or C; R = A or G; W = A or T; S = C or G; Y = C or T; K = G or T.

hybridomas that show binding property to DNA from an unimmunized MRL-*lpr/lpr* mouse (Park *et al.*, 1998). All of the monoclonal antibodies secreted from these hybridomas show higher affinities to dsDNA than ssDNA. In addition, they showed much higher affinities to DNA than to RNA (Park *et al.*, 1998). To test whether or not these anti-DNA antibodies show chain dominance in DNA binding, we first screened for the optimal oligonucleotide sequences to be used for different antibodies. We chose three different sequences (oligo 1, 2, and 3) for this purpose (Table 3). One of the representative results is shown in Fig. 1, which indicates that only the antibody G1-2 among the three antibodies tested has affinity to oligo-1. To examine the chain dominance of the antibodies, we performed the UV cross-linking experiment which measures the direct interaction of the H and/or L chains with DNA (Jang and Stollar, 1990). Representative results of the UV cross-linking experiment are shown in Fig. 2. In Fig. 2A, the upper and lower bands correspond respectively to the H and L chains of antibody G4-20 cross-linked to radio-labeled oligo-2. Therefore, both the H and L chains of the antibody G4-20 contribute (co-dominantly) to DNA binding. A single band in Fig. 2B represents the H chain of antibody G5-8 cross-linked to oligo-3. Therefore, in G5-8 antibody, the H chain is dominantly involved in DNA binding. To show the reproducibility of the experiment, cross-linking was done in four different wells of a 96-well plate. The resulting

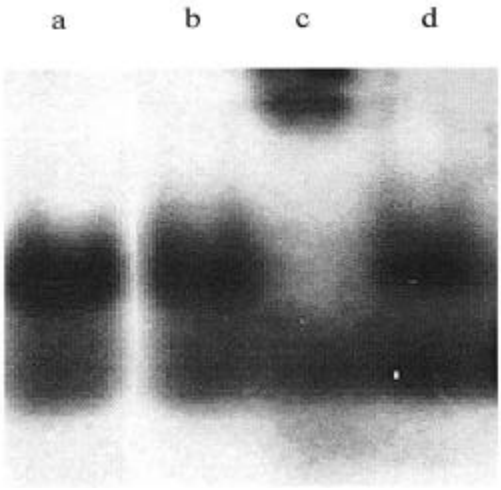

Fig. 1. Band mobility shift assay for the binding of 250 ng of radiolabeled oligo-1 by 10 g of each antibody. Lane a, migration of oligo-1 alone; lane b, migration of oligo-1 with G1-5; lane c, migration of oligo-1 with G1-2; lane d, migration of oligo-1 with G2-12.

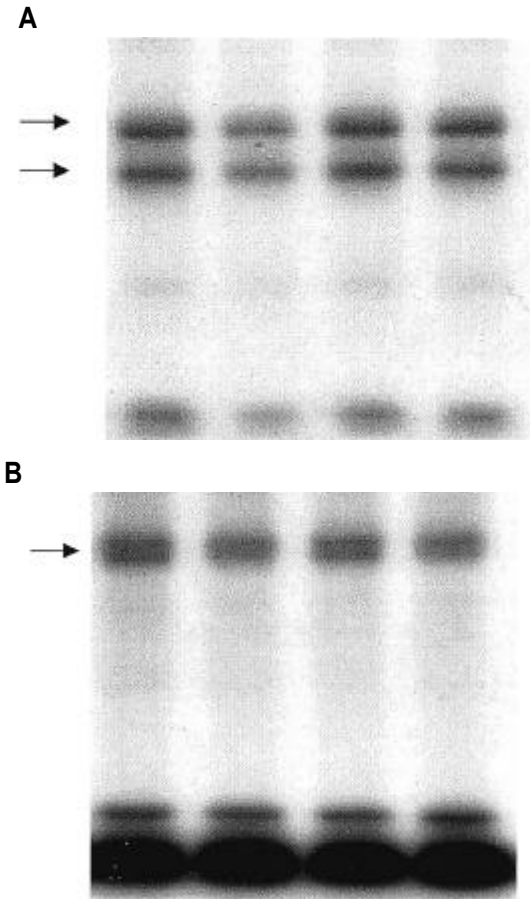

Fig. 2. Analysis of UV cross-linking of a monoclonal antibody with a labeled oligonucleotide by SDS-PAGE. **A.** Antibody G4-20 was mixed with γ-^{32}P(ATP) labeled oligo-2 in four wells of polystyrene plate and irradiated with short wavelength UV-light (254 nm) on ice. Irradiated mixtures were analyzed by 12% SDS-PAGE. The upper and lower arrows indicate the cross-linked H and L chains of G4-20, respectively. **B.** Antibody G5-8 was cross-linked with oligo-3. The arrow indicates the H chain cross-linked.

mixtures were analyzed side by side. The results of these experiments for all eight monoclonal antibodies are summarized in Table 3. As shown in Table 3, we could divide the monoclonal anti-DNA antibodies into two categories. The first category would include G5-8, G5-33, and G6-23 antibodies, which show H chain-dominance in DNA binding. The second category would include the rest of the antibodies (G1-2, G1-5, G2-12, G3-47, G4-20) in which H and L chains are co-dominantly involved in DNA binding.

Until now, only a few anti-DNA antibodies with H chain dominant properties in DNA binding have been reported. These antibodies include 3H9 (Radic *et al.*, 1991), Z22 (Polymenis and Stollar, 1995), and 2C10 (Jang *et al.*, 1996). From the structural and functional studies with these anti-DNA antibodies, it was suggested that the H chain is sufficient for antigen binding, and the L chain may modulate the binding selectivity. The results in this paper show that chain-dominance is not a rare phenomenon, reaching almost 40% (37.5%) of the antibodies that we isolated. In addition, we could not find the antibodies in which the L chain is dominantly involved in DNA binding.

Amino acid sequence analysis of the variable regions of the different anti-DNA antibodies To determine the critical factor(s) affecting the chain dominance of DNA binding by anti-DNA antibodies, we decided to compare amino acid sequences of the anti-DNA antibodies that belong to the two different categories. For this, nucleotide sequences of the V regions (both VH and VL) of all eight antibodies were determined. The results are shown in Figs. 3 and 4, and the usage of different gene families of V, D, and J fragments are summarized in Table 4. Among the H chain dominant antibodies, VL genes of G5-8 and G6-23 share the same VL and JL sequences, including the CDR3 regions (Fig. 3B). G5-33 has the same VL and JL sequences as G5-8 except one amino acid in FR (framework region) 1 (Fig. 3B). Although these antibodies (G5-8, G5-33, and G6-23) are using VH genes that are different from each other, they share exactly the same CDR3 sequences (Fig. 3A). These results suggest that there may have been a very strong selective pressure toward the usage of this particular CDR3 among the anti-DNA antibodies in *MRL-lpr/lpr* mice. As shown in Table 3, G5-8 and G5-33 have different sequence specificities in DNA binding. In a sequence analysis, the only difference in VH between these two antibodies lies in the four amino acids in the H chain FRs (Fig. 3A). This result suggests that the amino acid residues in FRs of VH sometimes affect the antigen specificity of an antibody, and the L chain of the anti-DNA antibodies does not always modulate

Table 3. Relative levels of UV cross-linking of the H and L chains of anti-DNA antibodies with oligonucleotides.

Name of clones	Oligonucleotides[a]	UV cross-linking (Mean% SD)		
		Heavy chain	Light chain	N[c]
G1-2	1	47±09	53±0.9	4
G1-5	2	49±0.5	51±0.5	4
G2-12	2	50±0.3	50±0.3	4
G3-47	1	50±0.3	50±0.2	4
G4-20	2	50±0.2	50±0.2	4
G5-8[b]	3	100±0.0	0±0.0	4
G5-33[b]	1	100±0.0	0±0.0	4
G6-23[b]	2	100±0.0	0±0.0	4

[a] Oligonucleotides are the most preferred ones for the binding by each anti-DNA antibody determined by band mobility shift assay. oligo-1, d(GAGAGAGAGAGAGAGAGAGA) · d(TCTCTCTCTCTCTCTCTCTC); oligo-2, d(GCGCGATATATATCGCGC) · d(GCGCGATATATATCGCGC); oligo-3, d(ATATAGCGCGCGCTATAT)d(ATATAGCGCGCGCTATAT).
[b] Bold letters represent the H chain dominant antibodies of which the L chain was not detected by autoradiogram.
[c] N represents the number of experiments repeated.

Table 4. [a]Family usage of gene fragments of the variable regions of anti-DNA antibodies.

Antibody	H chain domiance	VH			VL	
		V_H	D_H	J_H	V_L	J_L
G5-8	+	VH5	DQ52	JH3	Vλ1	Jλ1
G5-33	+	VH5	DQ52	JH3	Vλ1	Jλ1
G6-23	+	VH5	DSP2.8	JH3	Vλ1	Jλ1
G1-2	−	VH1	DSP2.8	JH3	Vκ21 group III	Jκ1
G1-5	−	VH1	DQ52	JH3	Vκ21 group III	Jκ1
G2-12	−	VH1	DSP2.8	JH3	Vκ21 group III	Jκ1
G3-47	−	VH1	DSP2.8	JH3	Vκ21 group III	Jκ1
G4-20	−	VH1	DSP2.8	JH3	Vκ21 group III	Jκ1

[a] Gene family usage was defined according to the determination in the web site of http://www.genetik.uni-koeln.de/cgi-bin/imgt/dnaplot scripts/vsearch.pl.

sequence specificity. In addition, the fact that the same VL is used in G5-8 and G5-33 indicates that these two antibodies may have been generated by either the somatic mutation of the H chain gene or the receptor editing process. Since the germ line VH sequence for this particular VH is unknown, we currently cannot draw a definite conclusion on this.

Among the H chains of G1-2, G1-5, G2-12, G3-47, and G4-20, in which both the H and L chains are co-dominantly involved in DNA binding, G1-2 and G1-5 have exactly the same VH and VL sequences except for one amino acid (Arg^{100} in G1-2 *vs* Phe^{100} in G1-5) in CDR3 of VL (Fig. 4B). Since the DNA-binding specificities of the two antibodies are different (Table 3), this is a good example of the L chains being responsible for DNA-binding specificity. Somatic mutation during the L chain gene rearrangement process may have caused the single amino acid difference between these two antibodies. G2-12 and G4-20 have exactly the same VH sequences even though their VL sequences are quite different (Fig. 4), indicating that they may have been derived from a single pre-B cell. The VH regions of the antibodies that belong to this category (co-dominant) shows almost the same amino acid sequences, even in the CDR3 regions except for a few silent mutations and amino acid changes in the FRs. Again, we cannot decide whether or not the sequence differences among the VH genes of these antibodies are the result of somatic mutations, or the usage of different germ line VH genes.

The H chain dominant antibodies utilize VH5 and JH3 gene segments with DQ52 or DSP2.8 segment. They all utilize Vλ1 and Jλ1 segments. However, the antibodies in the second category, in which both H and L chains are co-dominantly involved in DNA binding, utilize VH1, DSP2.8 and JH3 gene segments for VH, and Vκ21 group III and Jκ1 gene segments for VL. One exception is G1-5 in which DQ52 instead of DSP2.8 is used as a D gene segment.

Analysis of factors involved in chain dominance To explore the shared features of the primary structures that may determine chain dominance, we compared the CDR3 regions of both the H and L chains of anti-DNA antibodies belonging to the two categories described above. To draw a more general conclusion, the CDR3 sequences of several anti-DNA antibodies, which had been characterized in previous studies, were also compared. The antibodies, 2C10, Z22, and 3H9 were known to be H chain dominant in DNA-binding (Jang *et al.*, 1996, Polymenis and Stollar, 1995, Radic *et al.*, 1991). On the other hand, both the H and L chains are co-dominantly involved in DNA binding in antibody H241 (Jang and Stollar, 1990). As shown in

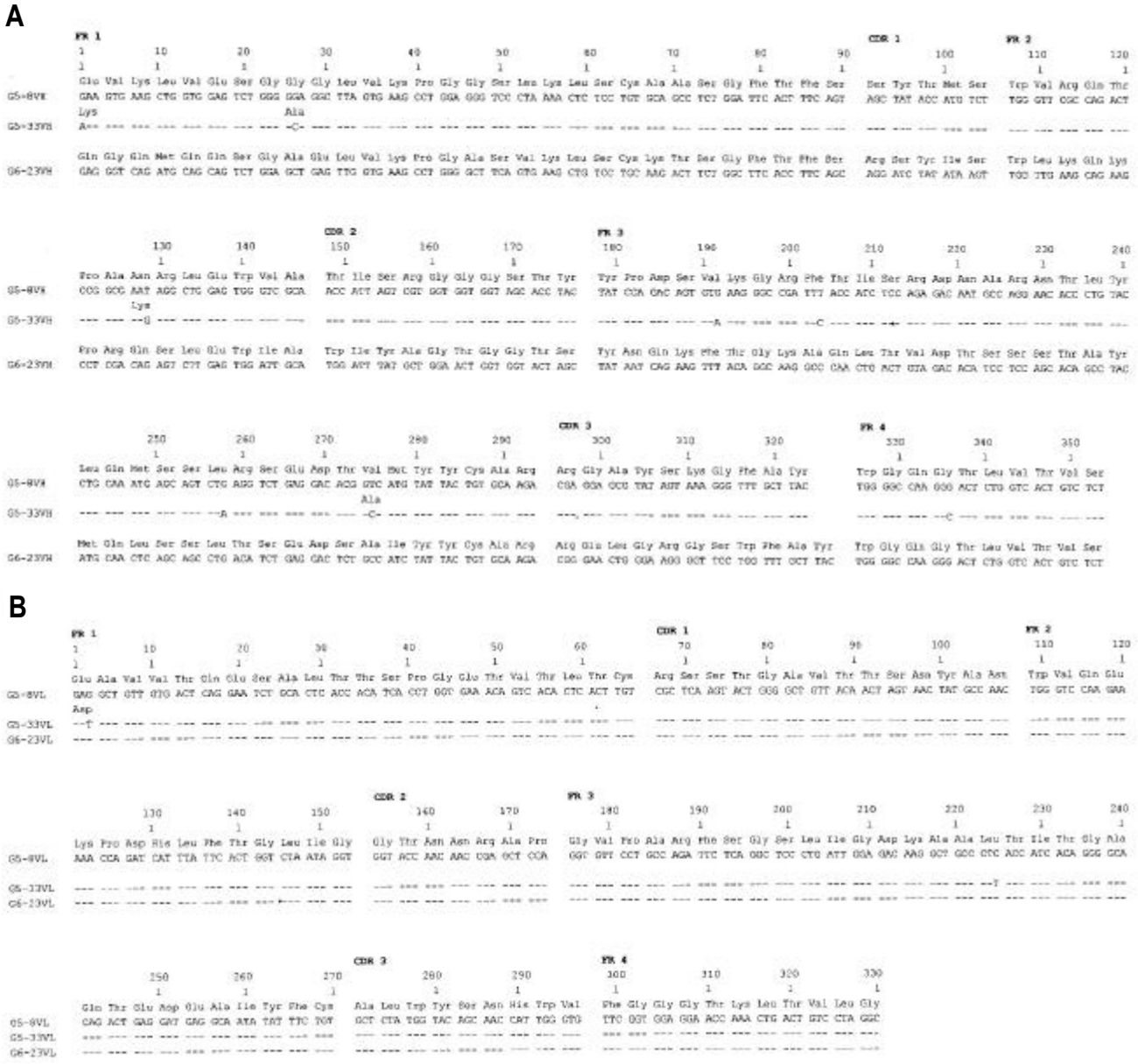

Fig. 3. A. Nucleotide sequences of VH regions of H chain dominant anti-DNA antibodies, G5-8 (GenBank Accession No. AF285276), G5-33 (GenBank Accession No. bankit348528), and G6-23 (GenBank Accession No. bankit348541). **B.** Nucleotide sequences of their VL regions; G5-8 (GenBank Accession No. bankit348524), G5-33 (GenBank Accession No. bankit348540), and 6-23 (GenBank Accession No. bankit348548). Sequences were determined by sequencing three to five clones from each antibody. Sequences that show high similarity among antibodies are aligned together. Identities are indicated by dashes. Gaps in the CDR3 region sequences of the VH are inserted to maximize sequence alignment.

Table 5, arginine residues can be found in CDR3s of all the H and L chains of the antibodies that are co-dominant in DNA binding. On the other hand, the same residue can be found in the CDR3s of the H chain, but not in the L chain, of the antibodies that are H chain dominant in DNA binding. Thus, we can conclude that the presence of arginine residue in the CDR3s determines whether or not a particular chain of an anti-DNA antibody would make contact with DNA. Another finding related to this is that the net charges of the VLs of the H chain dominant antibodies are significantly lower than those of the co-dominant antibodies (Table 5). The junctional variability resulting from imprecise joining of the VH coding sequences during VDJ recombination contributes diversity to the antigen receptors (Kim *et al*., 2000). In autoantibodies reactive with native DNA, an increased

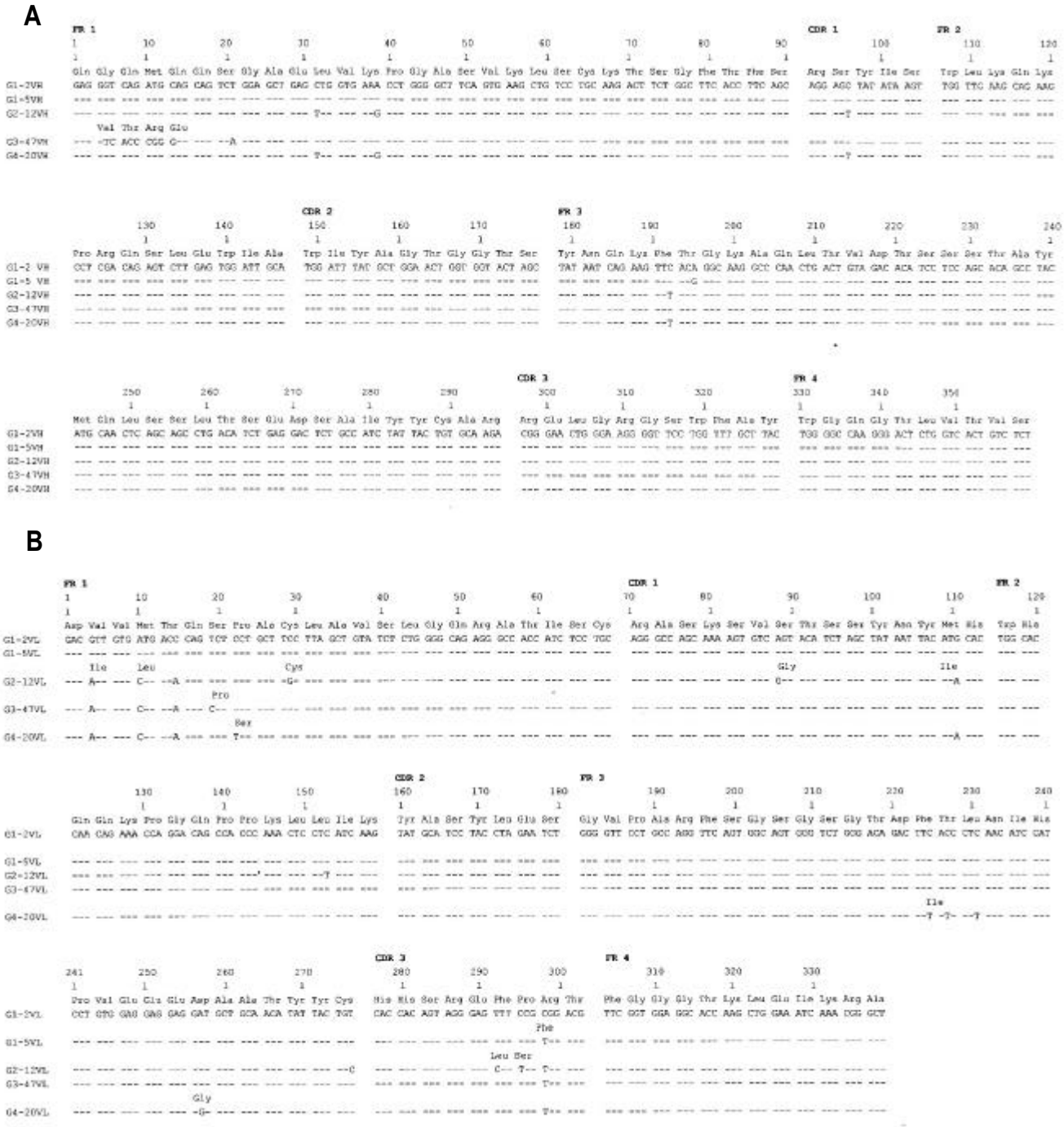

Fig. 4. A. Nucleotide sequences of VH regions of the H chain dominant anti-DNA antibodies, G1-2 (GenBank Accession No. bankit348557), G1-5 (GenBank Accession No. bankit348563), G2-12 (GenBank Accession No. bankit348572), G3-47 (GenBank Accession No. bankit348578), and G4-20 (GenBank Accession No. bankit348624). **B.** Nucleotide sequences of their VL regions; G1-2 (GenBank Accession No. bankit348561), G1-5 (GenBank Accession No. bankit348566), G2-12 (GenBank Accession No. bankit348575), G3-47 (GenBank Accession No. bankit348619), and G4-20 (GenBank Accession No. bankit348633). At least three clones from each antibody were sequenced. Identities are indicated by dashes. Gaps in the CDR3 region sequences of the VH are inserted to maximize sequence alignment.

frequency in the appearance of arginine in CDR segments, or in junctional regions of VH by somatic mutation, has been previously reported (Diamond *et al.*, 1992; Jang *et al.*, 1996; Marion *et al.*, 1992). However, from the results of this study, we suggest that the presence of arginine in CDR3 regions of the L chains is also important for the

Table 5. Comparison of amino acid sequences of VH and VL of H chain dominant and co-dominant anti-DNA antibodies.

A. H chain dominant antibodies.

	Amino acid sequence of CDR3		Number of arginines in CDR3		Net charge	
	VH-CDR3[e]	VL-CDR3	VH-CDR3	VL-CDR3	VH	VL
G5-8	**R R**GAYSKGFAY	ALWYSNHWV	2	0	+7	-1
G6-23	**R R**ELG**R**GSWFAY	ALWYSNHWV	3	0	+6	-1
2C10[a]	**R RR**Y**R**SSYAMDY	LQSDNMPLT	4	0	+6	-7
Z22[b]	**R** AYSNYGAMDY	QQYSKFPFT	1	0	+5	+3
3H9[c]	**R** A**R**SKYSYVMDY	QQWCGYPET	2	0	+6	+1

B. Co-dominant antibodies.

	Amino acid sequence of CDR3		Number of arginines in CDR3		Net charge	
	VH-CDR3[e]	VL-CDR3	VH-CDR3	VL-CDR3	VH	VL
G1-2	**R R**ELG**R**GSWFAY	HHS**R**EFP**R**T	3	2	+6	+8
G1-5	**R R**ELG**R**GSWFAY	HHS**R**EFPWT	3	1	+6	+7
G2-12	**R R**ELG**R**GSWFAY	HHS**R**ELSWT	3	1	+6	+7
G3-47	**R R**ELG**R**GSWFAY	HHS**R**EWPWT	3	1	+6	+7
H241[d]	**R** KNYGSSFDY	QHS**R**EFP	1	1	+6	+3

[a] The sequences of 2C10 are from Jang *et al.* (1996) and the GenBank access numbers for the VH and VL are U23046 and U23047, respectively.
[b] The sequences of Z22 are from Brigido and Stollar (1991) and the GenBank access numbers for the VH and VL are M60022 and M60020, respectively.
[c] The sequences of 3H9 are from Shlomchik *et al.* (1987) and the GenBank access numbers for the VH and VL are M18234 and M18237, respectively.
[d] The sequences of VH and VL of H241 are from Kofler *et al.* (1988) and Jang *et al.* (1996), respectively. Their numbers for GenBank access are M20831 and U23048, respectively.
[e] The first amino acid of VH-CDR3 is last amino acid of FR3.

direct contribution of the L chains in DNA binding. Although the H chain dominant property may be unusual in most antibodies in general, our results suggest that the H chain dominance is not a rare phenomenon among the anti-DNA antibodies. Also, the presence of arginine and net charges of V regions determine whether or not a particular chain would make direct contact with DNA. Currently, it is unknown if the two categories of the anti-DNA antibodies described in this study have different degrees of pathogenicity *in vivo*. Understanding the correlation between the biochemical properties of autoantibodies with their modes of antigen binding and the pathogenicity *in vivo* will advance our understanding of autoimmune diseases.

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