Independently derived Murine Glomerular Immune Deposit-forming Anti-DNA Antibodies Are Encoded by Near-identical V_H Gene Sequences

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Abstract

To examine the influence of variable region sequences on the capacity of individual lupus autoantibodies (autoAb) to form glomerular immune deposits, the complete V_H and V_L region sequences of three anti-DNA mAb that produced morphologically similar immune deposits after administration to normal mice were determined. The Ig were independently derived from 1-mo-old (H238, IgM), 3-mo-old (H8, IgG2a), and 6-mo-old (H161, IgG3) MRL-lpr/lpr mice, and they all produced subendothelial and mesangial immune deposits after passive transfer to normal mice. In addition, H238 and H161 produced granular deposits in small extraglomerular vessels. The mAb had nearly identical V_H gene sequences; H8 differed from H238 and H161 by a single nucleotide in FR1 that resulted in a histidine for glutamine substitution. This V_H gene sequence was also > 99% homologous to another anti-DNA Ab (termed H241), that we previously reported to produce glomerular immune deposits in a similar morphologic pattern. H161 and H238 were encoded by DFL16 and J_H2 genes, whereas H8 was encoded by a J_H4 gene. Different V_k family genes were used to encode the three mAb, however H161 and H238 both used a J_k5 gene. The results indicate that an identical or highly related V_H gene is used to encode a subgroup of murine lupus autoAb that share immune deposit forming properties. Furthermore, they raise the possibility that amino acid residues independent from those encoded by V_H genes may be influential in immune deposit formation at extraglomerular sites. (*J. Clin. Invest.* 1993. 91:402–408.) Key words: anti-DNA antibody • systemic lupus erythematosus • V_H gene • autoimmunity • glomerulonephritis

Introduction

The influence of specific V gene sequences on the production of nephritogenic lupus autoantibodies (autoAb)[1] has been the focus of our research. Selective V gene use among pathogenic Ig is suggested by the observation that properties associated with the antigen (Ag) binding region distinguish nephritogenic Ig from their serum counterparts. In this regard, we and others have demonstrated that Ig eluted from glomeruli of individuals with active nephritis are enriched for anti-DNA and other autoantigen (autoAg) binding properties (1–7). In comparison to serum Ig, nephritogenic Ig are highly cross-reactive; individual Ig eluted from individuals with lupus nephritis bind to multiple autoAg, including cell surface and basement membrane antigens normally present within the glomerulus (3, 4, 8). The presence and relative concentration of Ig with shared idiotypes among nephritogenic Ig provides further support for an influential role of the Ag binding region in the formation of glomerular immune deposits (3–5, 9, 10).

To address this issue directly, we reasoned that it would be useful to determine the V gene sequences of autoAb with nephritogenic properties. For purposes of identifying nephritogenic autoAb, the capacity of individual MRL-lpr/lpr–derived monoclonal anti-DNA Ab to produce glomerular immune deposits in normal mice was determined (11). In these initial studies, the monoclonal autoAb were selected on the basis of Ag binding properties shared with Ig eluted from the kidneys of mice with active nephritis; the selected mAb were subsequently administered to normal mice. Consequently a subset of monoclonal anti-DNA Ab were identified that reproducibly formed glomerular immune deposits. Particularly noteworthy, the location of immune deposits within glomeruli varied and was dependent on the administered monoclonal anti-DNA Ab, and this was associated with different disease patterns. Furthermore many Ig did not produce immune deposits. These observations indicate that not all anti-DNA Ab are pathogenic, and they raise the possibility that the Ab V region influences both the capacity of individual autoAb to form immune deposits and the site of immune deposit formation within the glomerulus.

A relevant question that evolved from these studies is: Are autoAb that produce similar patterns of glomerular immune deposits encoded by related V genes? To address this question, in the present studies, the heavy and light chain V gene sequences of three monoclonal anti-DNA Ab that produced mesangial and subendothelial immune deposits after transfer to normal mice were determined. These particular monoclonal anti-DNA Ab were selected, because they produced morphologically similar patterns of immune deposits. Furthermore, to exclude the potential bias of examining clonally related Ig from an individual mouse, the Ig were intentionally selected from three MRL-lpr/lpr mice of different ages (12).

Methods

Selection of mAb. As indicated above, our aim was to determine the V region sequences of mAb that reproducibly produced glomerular immune deposits after passive transfer to normal mice. For this purpose, monoclonal anti-DNA Ab were selected from a large panel of hybridomas derived from MRL-lpr/lpr mice of varying ages (12), on the basis of shared cross-reactive Ag binding properties with Ig eluted from the kidneys of 3–4-mo-old MRL-lpr/lpr mice with active nephritis (3). After passive transfer, a subset of monoclonal anti-DNA Ab reproducibly produced persistent glomerular immune deposits when adminis-

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1. *Abbreviations used in this paper:* Ab, antibodies; Ag, antigens.

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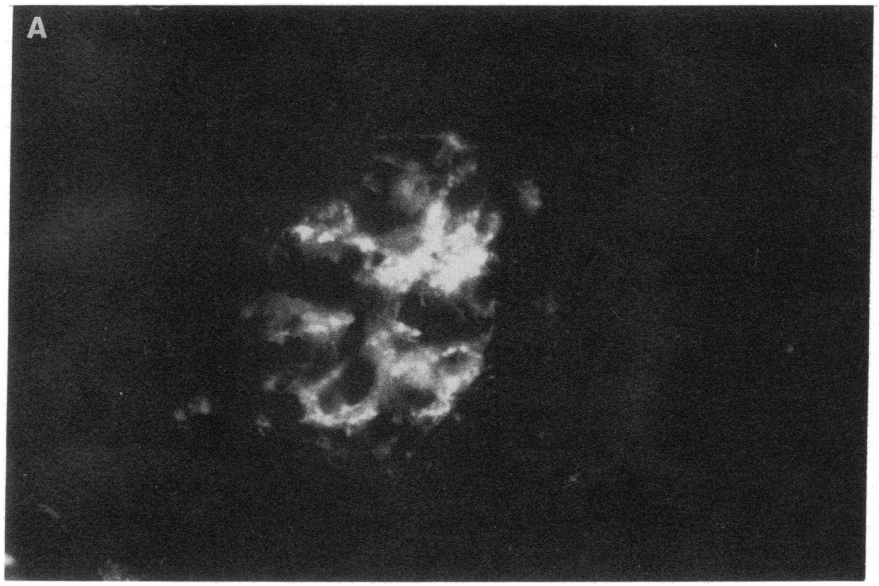
tered as hybridomas and as purified Ig to normal (AKR × DBA)2 mice (11). This recipient strain was selected because of histocompatibility with both the MRL-lpr–derived B cells and the NS-1 fusion partner used to produce the hybridomas. Noteworthy: (*a*) the administered anti-DNA Ab was the only detectable Ig within the lesions; (*b*) the location of the glomerular immune deposits varied with the administered antibody; and (*c*) the different sites of immune deposit formation were associated with distinct histologic and clinical profiles. Furthermore, there were monoclonal anti-DNA Ab that either did not produce immune deposits or produced only minimal mesangial deposits (direct immunofluorescence), unassociated with light or electron microscopic abnormalities. From the group of immune deposit–forming Ab, three (termed H8, H161, and H238) were selected for further analysis because they produced an indistinguishable histological pattern characterized by persistent mesangial and subendothelial immune deposits (as determined by immunofluorescence and electron microscopy). Two of the mAb (H161 and H238) also produced vascular immune deposits, whereas the other (H8) did not. The mice that received the H238 (IgM) hybridomas developed proliferative glomerulonephritis and proteinuria, and the H8 animals developed proteinuria. A typical example of the immune deposits produced by these mAb is illustrated in Fig. 1. The in vivo and in vitro characteristics of these three mAb are summarized in Table I.

V gene sequence determination and analysis. Total RNA was isolated from each B cell hybridoma by the guanidium/CsCl method as previously described (13). Full-length cDNA was synthesized from 80 μg of total RNA by primer extension using avian myeloblastosis virus reverse transcriptase (Boehringer-Mannheim, Corp., Indianapolis, IN), RNase H (Bethesda Research Laboratories, Gaithersburg, MD), DNA polymerase 1 (NEN) and either Cγ, Cu, or Ck primers including 5′(GGCCAGTGGATAGAC)3′, (IgG2a); 5′(GACCAAGGGATAGAC)3′, (IgG3); 5′(CAGGAGACGATGGGGGA)3, IgM; and



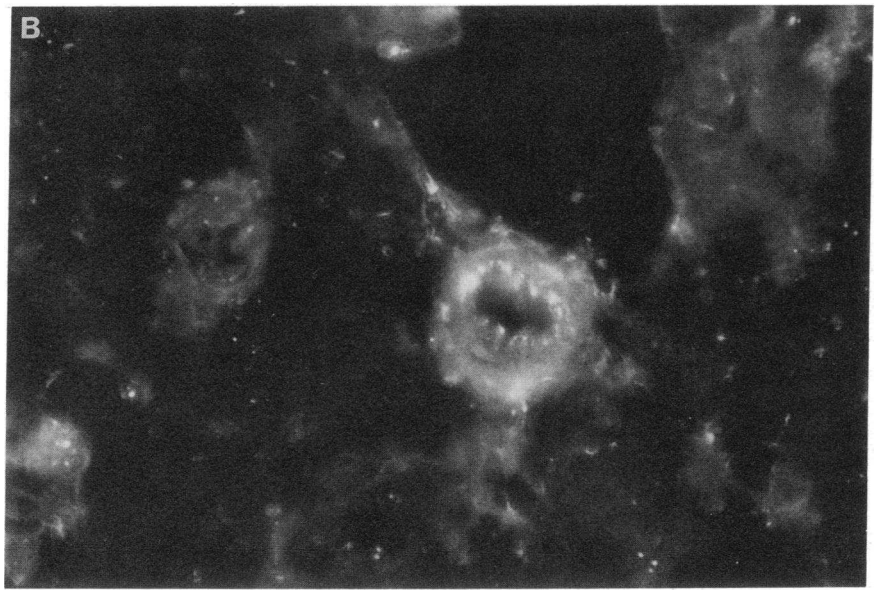


Figure 1. (*A*) Glomerular immune deposits after administration of H8 hybridomas to normal mice; direct immunofluorescence (×400). Immune deposits were visualized in a capillary wall and mesangial distribution as previously reported for H161 and H238. (*B*) Immune deposits within small vessels after the administration of H161 to normal mice. Direct immunofluorescence (×400). H238 also produced vascular immune deposits, whereas H8 did not.

Table I. Characteristics of MRL 1pr/1pr Immune Deposit-forming mAb

mAb	Age	Isotype	Antigen binding properties*					U_P	Immune deposits	
			V_H	ssDNA	dsDNA	SmRNP	Laminin	mg/day	Glo	Vas
8	3 mo	IgG2a	J558	++	++	++	−	1.5 ± 0.4	mes/subend	−
161	6 mo	IgG3	J558	+	−	+++	−	1.0 ± 0.2	mes/subend	+
238	1 mo	IgM	J558	+	−	−	+	1.9 ± 0.6	mes/subend	+

* As determined by ELISA: scale = minimum concentration of mAb that produced OD405 > 0.500, when positive control mAb reading was between 1.0 and 1.2 and negative control mAb < 0.05; +++ = <0.1 μg/ml; ++ = 0.1–1.0 μg/ml; + = 1–10 μg/ml; − = >10 μg/ml. Age indicates age of mouse from which hybridoma was derived. Immune deposits refers to the pattern of immune deposit formation after administration to normal mice. Glo, glomerular; Mes, mesangial; subend, subendothelial; and Vas, vascular. V_H refers to V_H gene family use by the mAb. U_P, quantitation of proteinuria/24 h; the level (mean ± S.D.) for the normal 4–6 wk female (AKRxDBA2)F1 mice was <1.0 mg/d.

5′(CTGCTCACTGGATGGTGGGA)3′, V_k (13). The cDNA was blunt ended with T4 DNA polymerase and ligated into the SmaI-digested M13mp18 RF (NEBL) as described (13). The M13 RF was transformed into DH5-aF′IQ (Bethesda Research Laboratories). Colonies were screened with chain specific ^{32}P-labeled oligoprobes, including 5′(TGGGGCTGTTGTTTT), IgG2a; 5′(TGGGGCTGTTGTTGT), IgG3; 5′(ATTTGGGAAGGACTGAC)3′, IgM; and 5′(AGATGGATACAGTTGGT), V_k. Positive plaques were subcloned, purified and sequenced by incorporation of dideoxynucleotides using the Sequenase Version 2.0 Kit (United States Biochemical Corp., Cleveland, OH). The sequences of at least two distinct cDNA clones containing complete V region sequence were determined for each Ig chain. Sequences were analyzed using the MacVector program and GenBank.

Results

The nucleotide and deduced amino acid sequences of the heavy chain variable region genes of H8, H161, and H238 are illustrated in Fig. 2. There is identity in the V_H gene for H161

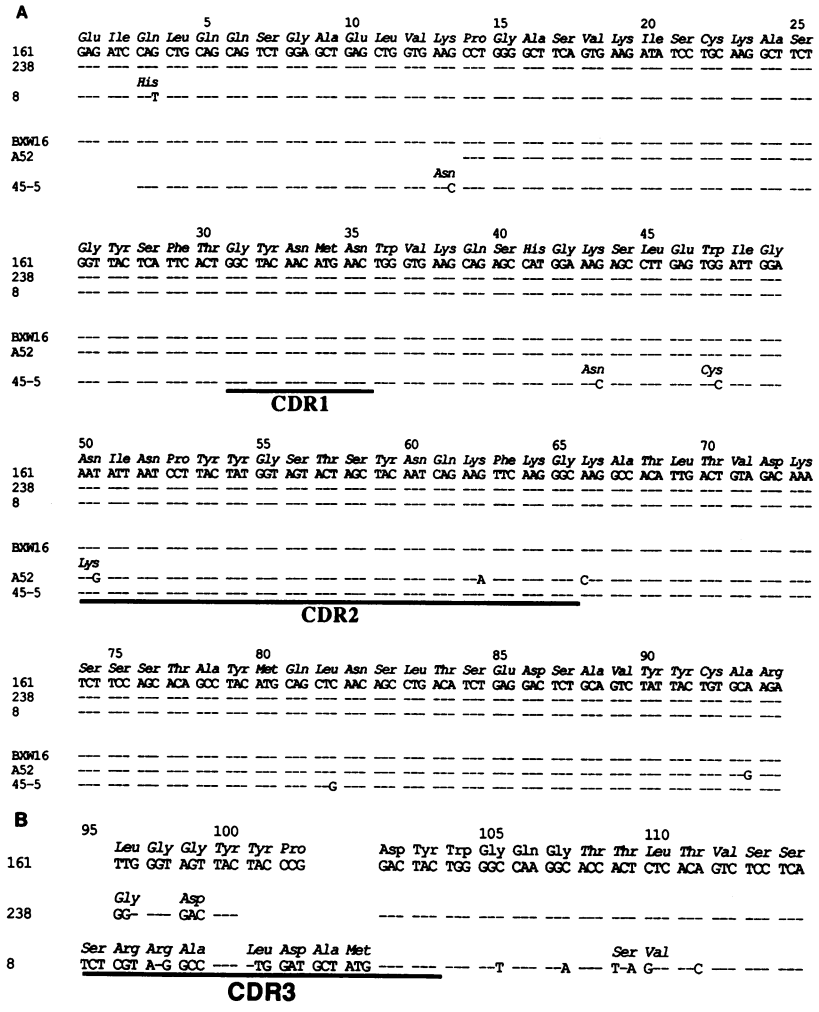


Figure 2. Nucleotide and deduced a.a. sequences of the heavy chain variable region genes of H161, H238, and H8. In this and subsequent figures, the dashes indicate identity with the residue given in the top line. Codon numbering is according to Kabat et al. (14); CDR are underlined. (*A*) V_H gene sequences. In addition, the V_H gene sequences of three anti-DNA Ab, BXW16 (15), A52 (16), and 45-5 (17) identified by GenBank search that showed ≥ 99% homology with V_H H161, H238, and H8 are illustrated. BXW16 is a (NZB × W)F1 IgM mAB, A52 a (NZB × W)F1 IgG2b mAb and 45-5, an MRL 1pr IgG2a mAb. (*B*) D_H and J_H gene sequences.

and H238, and H8 differs only by a histidine for glutamine substitution in FR1. H161 and H238 were encoded by a DFL16 gene, whereas H8 used a DSp2 gene. H161 and H238 were encoded by the J_H2 gene, whereas H8 was encoded by the J_H4 gene.

The nucleotide and deduced amino acid sequences of the light chain variable region genes of H8, H161, and H238 are illustrated in Fig. 3. The three mAb are encoded by different V_k family genes. H161 and H238 use identical J_k5 genes, whereas H8 is encoded by a J_k2 gene.

Discussion

The results of these studies establish a relationship between a specific V gene sequence and the glomerular immune deposit–forming capacity of a subgroup of monoclonal anti-DNA Ab. The significance of these observations is emphasized by the experimental strategy used to select the Ig (11, 27). They were independently derived from different mice, thus eliminating the use of clonally related Ab from an individual animal. In addition, they all shared properties with nephritogenic Ig; they cross-reacted with multiple autoAg. Most importantly, they all produced mesangial and subendothelial immune deposits after passive transfer to normal mice (11). The use of the J558 V_H gene family to encode the three monoclonal anti-DNA Ab is in accord with our previous observations that Ig encoded by V_H genes from this family may be disease relevant (12), and it is consistent with the possibility that there were shared V region sequences among the group.

A structure-function relationship is suggested by the near homology of the V_HH8, V_HH161, and V_HH238 sequences. Additionally, a fourth monoclonal anti-DNA Ab, termed H241, that we previously observed to produce mesangial and subendothelial immune deposits that were similar to those produced by the present trio (28) was recently found to use a nearly identical V_H gene sequence (29). This homology is particularly noteworthy because all four anti-DNA Ab (H8, H161, H238, and H241) have distinguishable CDR3 regions, and they are encoded by different V_L genes (29). Additional disease relevance of this V_H gene sequence is suggested by the recent report of Singh and co-workers that the H241 V_H germline gene is present in multiple autoimmune strains and absent in many normal strains (30).

Identification of a specific V gene sequence associated with a distinct pattern of glomerular immune deposit formation is especially remarkable, given the criteria used to select this group of autoAb. Although anti-DNA autoAb are frequently encoded by J558 genes, this represents the largest V_H gene family, most likely including > 100 individual genes (31, 32). In this regard, multiple different individual V_H genes have been observed to encode anti-DNA Ab, including those from both J558 and other V_H gene families (12, 18, 19, 33–36). The pathogenic relevance of this particular V_H gene is emphasized by comparison to other previously published murine V_H gene sequences. A few points warrant emphasis. The most closely related V_H genes were used to encode anti-DNA Ab from lupus mice (Fig. 2 *A*). The nephritogenicity of this latter group of autoAb has not been examined, however, in vivo analysis would be of particular interest, since both the V_H CDR3 region and the V_L genes are different from those used by the present group. The results should provide additional information about the influence of regions distinct from the V_H gene on immune deposit formation. It is highly probable that variations in these areas may either facilitate or inhibit the glomerular immune deposit formation (29, 37).

It is also noteworthy that this particular J558 V_H gene is different from many other previously reported V_H genes used to encode anti-DNA Ab, including those derived from both MRL and other autoimmune strains (15–19, 37–39). In this regard, we and others have observed that autoAb that either produce immune deposits in locations distinct from those described in the present study or do not produce immune deposits use different V_H gene sequences (11, 13, 27, 37, 39, 40, 41, and unpublished observations). This includes both autoAb encoded by J558 genes and autoAb encoded by genes that belong to different V_H gene families. The protein sequences of prototypic J558 encoded anti-DNA Ab derived from representative MRL anti-DNA Ab, including a J558 encoded Ig that did not produce immune deposits, illustrate these differences (Fig. 4). Of additional interest, the presence of two arginines in the CDR3 region of H8 is consistent with the conclusions of Schlomchik and co-workers that this motif confers anti-dsDNA Ab activity (19). Nevertheless, this motif was not necessary for immune deposit formation; there were no cationic amino acids (His, Arg, or Lys) in the CDR3 regions of either H238 or H161, and only 2/22 amino acids were cationic in the V_H encoded CDR1 and CDR2 regions combined. Structural studies, in progress, should help determine the precise conformational requirements necessary for immune deposit formation.

Other V gene segments, distinct from this V_H gene, also appear to influence both the location of immune deposit formation and the Ag binding properties of this group of anti-DNA Ab. Accordingly, H238 and H161, the two monoclonal anti-DNA Ab with the most similar CDR3 regions and identical J_H regions, both produced extraglomerular deposits in small vessels, whereas these deposits were not observed with either H8 or H241 (28). Differences in the CDR3 region and V_L sequences also resulted in variations of the autoAg binding profiles of the MRL mAb. The influence of these segments (CDR3 and V_L) on Ag binding is also supported by comparison to other Ig with related V_H gene sequences (Fig. 2); variation in autoAg binding specificities among these Ig have been observed (15–17).

The V gene sequence homologies also have important implications regarding the genetic origins of pathogenic autoAb in murine lupus. The use of germline encoded V region sequences for immune deposit forming autoAb is strongly suggested by the identity of the V_H gene sequences of the independently derived MRL mAb H161 and H238, and of the (NZB × NZW)F1-derived mAb BXW16 and A52 (Fig. 2 *A*). The H8 V_H gene differs by a single nucleotide substitution in FR1. Additionally, the H161 light chain appears to be encoded by V_L and J_L gene sequences that are identical to the BALB/c germline (20). Precisely how recombinatory and/or somatic events influence the immune deposit forming capacity of these autoAb cannot be ascertained from this initial analysis, however, we plan to evaluate the role of these processes in future experiments. A related issue is the actual number of individual V genes used to encode pathogenic lupus autoAb. The present results, interpreted in the context of other studies, suggest that V gene usage among autoAb may be more limited than initially perceived (13, 15, 19, 37, 38, 42). This is not totally unexpected, because the antibodies were selected on the basis of

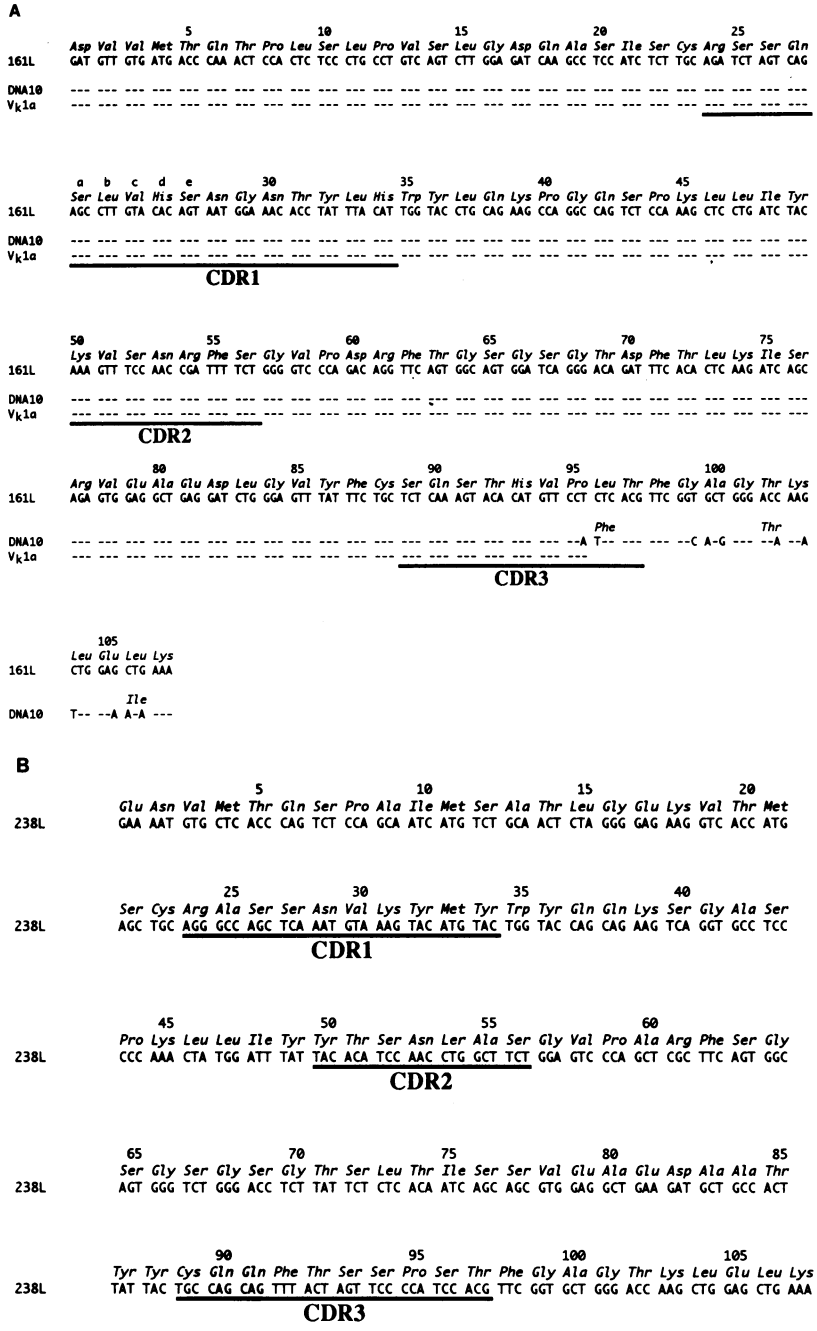


Figure 3. Nucleotide and deduced a.a. sequence of the light chain genes of H161 (*A*), H238 (*B*), and H8 (*C*). The mAb were encoded by different V_k families: H161, V_k1; H238, V_k4 and H8, V_k8. Antibodies encoded by V_L sequences identified by GenBank that showed the greatest homology with the anti-DNA Ab include (*A*) H161. DNA10 a MRL-lpr/lpr anti-DNA Ab (15) and V_k1a, a BALB/c germline gene (20). Not shown are DNA4, a MRL-lpr/lpr anti-DNA Ab (15); V105, a BALB/c myeloma kappa chain (21); 29-IG5, a BALB/c anti-GAT Ab (22); and GP138-10, a MRL-lpr/lpr–derived Ab (17) all sharing > 99% homology. (*B*) H238. The most homologous Ig (not shown) were AN09K, a BALB/c-derived anti-DNP Ab (23); and V_5-J_3, a nonproductively rearranged V_k4 gene derived from an NZB mouse sharing 95% homology (24). (*C*) H8. AM13 (19) an MRL-lpr/lpr–derived rheumatoid factor. Not shown are H210-56, a BALB/c-derived anti Influenza HA Ab (25) and 180.2G6, a BALB/c-derived antiphosphocholine Ab (26) with 96.5% homology.

their Ag binding specificities (i.e., anti-DNA). Nevertheless, the observed diversity in disease expression among J558 encoded anti-DNA Ab indicates that relatively subtle differences in the Ag binding region have profound effects on nephritogenicity.

This conclusion is also supported by previous evaluation of the relative contribution of variable region segments to the pathogenic potential of lupus autoAb. Reininger and coworkers demonstrated the importance of the heavy chain of an MRL-lpr/lpr–derived monoclonal rheumatoid factor (cryoglobulin) that transferred glomerulonephritis and cutaneous vasculitis to normal mice (40). Hybrid molecules, consisting of the original heavy chain associated with a novel light chain, retained cryoglobulin activity and induced glomerulonephritis, but did not produce vasculitis, supporting an influential role for the Ag binding region in disease expression. Using a related approach, Radic and co-workers observed variability in the anti-DNA activity of an MRL-lpr/lpr–derived mAb (43). A requirement for specific combinations of V gene segments for the production of pathogenic potential of lupus autoAb is consistent with the observations of Tsao and co-workers. They observed that the V_H gene encoding a (NZB × NZW)F1-derived pathogenic anti-DNA Ab was nearly identical to the V_H gene of a nonpathogenic MRL-lpr/lpr–derived anti-DNA Ab, however the other V region segments were distinct from one another (37).

It is evident from these reports that different V_H genes may be used to encode pathogenic autoAb. This conclusion is also

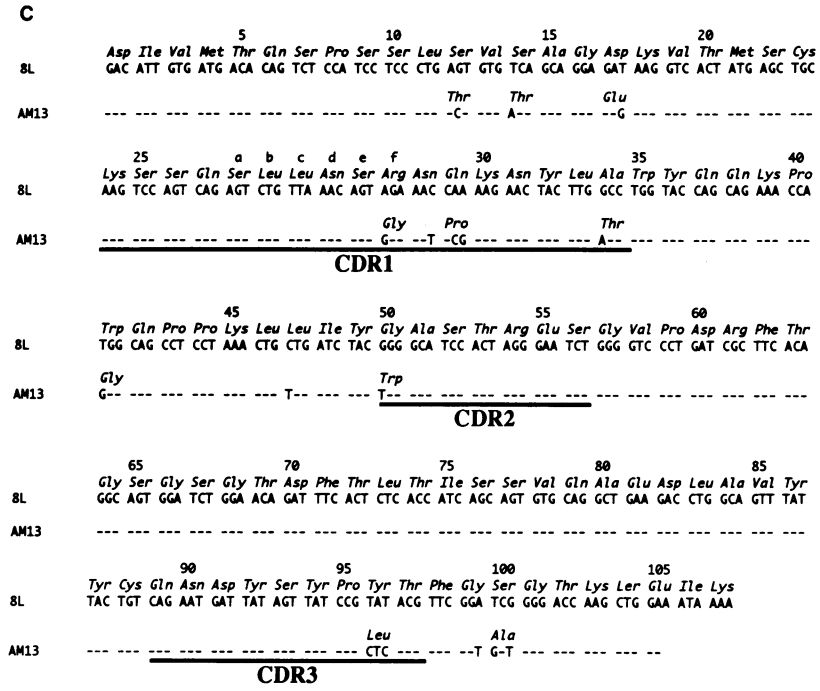


Figure 3 (Continued)

supported by our previous observations in SNF1 mice. Although three clonally related anti-DNA antibodies all produced glomerular immune deposits and glomerulonephritis after passive transfer to predisease SNF1 mice, their V_H gene was not highly related to the V_H gene sequence of the nephritogenic Ig described above (11, 39). Different mechanisms of immune deposit formation may account for the observed disparities. In this regard, we previously reported that morphologically distinct lesions developed after the administration of individual anti-DNA Ab to normal mice (11). Alternatively, although the primary sequences of the latter autoAb are different from those reported herein, similar conformationally dependent residues within the Ag binding regions of the individual autoAb could serve to interact with the same ligand to initiate immune deposit formation. Structural analysis of both the pathogenic autoAb and the relevant target antigens should provide clues to the mechanisms underlying these pathologic processes.

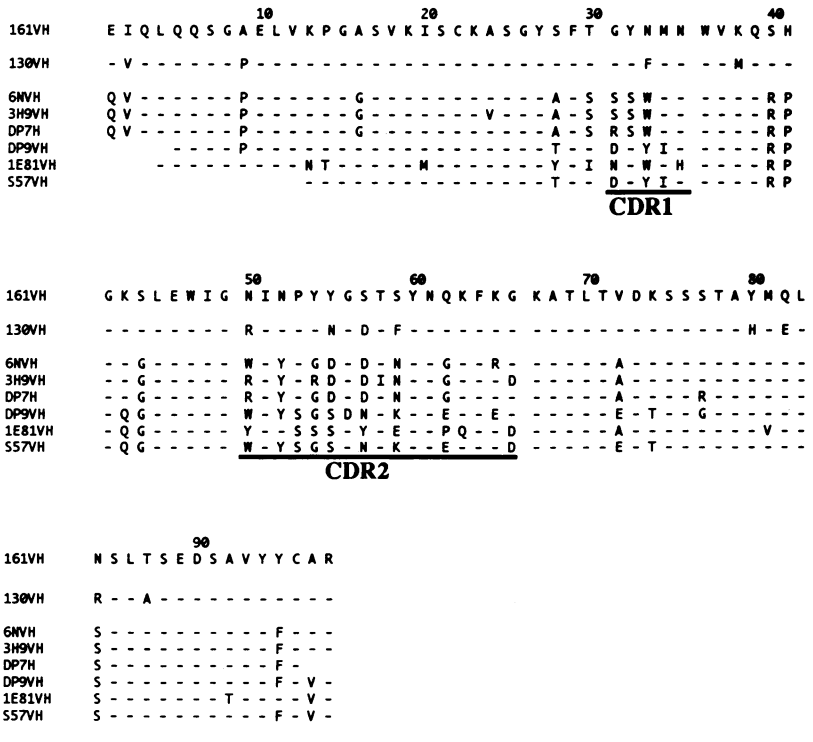


Figure 4. Comparison of deduced amino acid sequence of V_HH161 to H130, a nonpathogenic MRL-lpr/lpr anti-DNA Ab (18), and representative J558 encoded anti-DNA Ab from a large panel of MRL lpr/lpr Ab from multiple mice (19). Each mAb. within the latter panel is representative of a clonally expanded population from an individual mouse.

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