Ligand Recognition by Murine Anti-DNA Autoantibodies

II. Genetic Analysis and Pathogenicity

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Abstract

Although anti-DNA autoantibodies are an important hallmark of lupus, the relationships among anti-DNA structure, reactivity, and pathogenicity have not been fully elucidated. To further investigate these relationships, we compare the variable genes and primary structure of eight anti-DNA mAbs previously obtained from an MRL/MpJ-*lpr/lpr* mouse along with the ability of three representative mAbs to induce nephritis in nonautoimmune mice using established adoptive transfer protocols. One monospecific anti–single-stranded (ss) DNA (11F8) induces severe diffuse proliferative glomerulonephritis in nonautoimmune mice whereas another anti-ssDNA with apparently similar in vitro binding properties (9F11) and an anti–double-stranded DNA (4B2) are essentially benign. These results establish a murine model of anti-DNA–induced glomerular injury resembling the severe nephritis seen in lupus patients and provide direct evidence that anti-ssDNA can be more pathogenic than anti–double-stranded DNA. In vitro binding experiments using both protein–DNA complexes and naive kidney tissue indicate that glomerular localization of 11F8 may occur by recognition of a planted antigen in vivo. Binding to this antigen is DNase sensitive which suggests that DNA or a DNA-containing molecule is being recognized. (*J. Clin. Invest.* 1996. 97:1748–1760.) Key words: autoimmunity • gene • glomerulonephritis • lupus • wire-loops

Introduction

The presence of anti-DNA autoantibodies (anti-DNA)[1] in the serum of patients afflicted with SLE is a clinical hallmark of this disorder (1, 2). Although anti-DNA are involved in the renal pathogenesis of SLE (3–5), a clear understanding of the events leading from anti-DNA production to kidney damage has not yet been fully elucidated (6). An early model to explain lupus nephritis suggested that circulating anti-DNA–DNA immune complexes deposit within glomeruli (4, 5), but both DNA and anti-DNA–DNA immune complexes are rapidly cleared from circulation indicating that other mechanisms of glomerular localization are probably operative (7–10). More recently, in vitro binding experiments have shown that anti-DNA can form complexes with DNA or protein–DNA complexes trapped within glomeruli and that anti-DNA can bind extracellular matrix components which comprise the normal glomerular basement membrane (GBM; 11–20). These studies have led to the "planted antigen" and "cross-reactivity" hypotheses, respectively (reviewed in reference 6). While all these hypotheses differ, and may not be mutually exclusive, they each require an essential first step: the binding of a pathogenic anti-DNA to its antigen. Hence, determining the ligand binding properties and variable (V) region structure of anti-DNA is important for understanding the renal pathogenesis of SLE.

The binding properties and genetic origins of several hundred monoclonal anti-DNA (mAbs) from lupus-prone mice have been characterized (1, 2, 21–23). By contrast, very few of these mAbs have been tested for their ability to induce nephritis in vivo. In fact, only about 27 anti-DNA mAbs are known to be pathogenic (pathogenic mAbs being defined here as those that induce glomerulonephritis of any severity as judged by renal biopsy and histologic evaluation; 11–16, 23–34). Consequently, patterns of ligand recognition, physicochemical properties like pI, isotype, or idiotype, and V-region gene structures that can unambiguously distinguish pathogenic and nonpathogenic anti-DNA have not yet been established (reviewed in references 6, 24).

In previous work, we generated several anti-DNA mAbs from an unmanipulated MRL/MpJ-*lpr/lpr* (MRL-*lpr*) mouse and characterized their binding affinity and specificity at equilibrium, their mode of DNA recognition, and the molecular interactions used for DNA binding (35). Here we report an analysis of the V genes that encode eight of these mAbs and the results of adoptive transfer experiments to determine the ability of representative clones to induce nephritis in nonautoimmune mice. Four mAbs specific for just single-stranded DNA (ssDNA) are clonally related, including three (9F11, 15B10, and 15D8) that share similar affinity and specificity. Another monospecific anti-ssDNA, 11F8, apparently possesses similar in vitro binding properties to the clonally related mAbs and is encoded by different V genes. Of particular interest, 11F8 induces acute diffuse proliferative glomerulonephritis when adoptively transferred into nonautoimmune mice whereas 9F11 and an anti–double-stranded (ds) DNA (4B2) are essentially benign. This latter result is significant because it establishes a murine model of anti-DNA–induced glomerular injury

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1. *Abbreviations used in this paper:* anti-DNA, anti-DNA autoantibodies; ctDNA, calf thymus DNA; dsDNA, double-stranded DNA; GBM, glomerular basement membrane; MRL-*lpr*, MRL/MpJ-*lpr/lpr*; ssDNA, single-stranded DNA; V_H, heavy chain variable region; V_L, light chain variable region.

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that resembles the severe nephritis seen in many lupus patients. Furthermore, it provides direct evidence that an anti-ssDNA mAb can be more pathogenic than anti-dsDNA (both 4B2 and other anti-dsDNA that are nephritogenic; 11–16, 23–34). In vitro binding experiments using both protein–DNA complexes and naive kidney tissue indicate that glomerular localization of 11F8 may occur by recognition of a planted antigen in vivo. Binding to this antigen is DNase sensitive which suggests that DNA or a DNA-containing molecule is being recognized.

Methods

Anti-DNA. Anti-DNA mAbs were derived from a 26-wk-old MRL-*lpr* mouse. The purification and characterization of these mAbs has been described previously (35).

DNA. DNA primers were synthesized on an Expedite Nucleic Acid Synthesizer (Milligen, Framingham, MA) using β-cyanoethyl phosphoramidite chemistry. Oligomers were deprotected and purified using OPC columns (ABI, Foster City, CA) according to the manufacturer's protocols. The preparation of ssDNA and dsDNA for use in ELISA has been described previously (35).

Cloning and sequencing of variable heavy (V_H) and light chain (V_L) genes. Poly(A^+) RNA was isolated from 10^7 hybridoma cells by adsorption to poly(dT) cellulose using the FastTrack mRNA isolation system (Invitrogen Corp., San Diego, CA). Full length first-strand cDNA was obtained by reverse transcription of poly(A^+) RNA using an oligo(dT) primer and avian myeloma reverse transcriptase using the cDNA Cycle kit (Invitrogen Corp.). Anti-DNA V_H and V_L genes (along with C_H1 and C_L) were amplified by PCR from first strand cDNA using AmpliTaq polymerase (Perkin Elmer Cetus, Norwalk, CT). A degenerate set of primers was used to amplify the V_H genes whereas a single primer set was used to amplify the V_L sequences. The sequence of the primers is as follows (Immunozap Cloning Kit; Stratacyte, La Jolla, CA):

V_L(κ) GTGCCAGATGTGAGCTCGTGATGACCCAGTCTCCA (3′)
C_L(κ) TCCTTCTAGATTACTAACACTCTCCCCTGTTGAA
C_H^1 [γ2a2b] GATATCACTAGTGGGCCCGCTGGGCTC
C_H^1 [γ3] TGGGCAACTAGTACCTGGGGGGGTACTGGGCTTGG
V_H AGGTCCAGCT(T/G)CTCGAGTC(T/A)GG

Briefly, 10–100 ng of cDNA was added to PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin) containing dNTPs (each 250 μM), primers (each 1 μM), and AmpliTaq polymerase (5 U) to a final vol of 100 μl. The samples were overlaid with liquid wax (80 μl, MJ Research, Watertown, MA), denatured (94°C, 5 min), and annealed (54°C, 5 min) before being subjected to 30 cycles of extension (72°C, 3 min), denaturation (93°C, 1.5 min), and annealing (54°C, 2.5 min) using a thermal cycler (MJ Research). The length of the PCR products was verified on a 1% agarose gel.

PCR products were ligated into the precut sequencing vector pCRII (Invitrogen Corp.) using T4 DNA ligase (4 U, 15°C, 18 h) and the construct was used to transform competent *Escherichia coli* INVαF′ cells (Invitrogen) according to the manufacturer's protocol. Transformants were spread on LB agar plates containing both ampicillin (50 μg/ml) and X-Gal (10 mg/plate) and were grown overnight at 37°C. Colonies containing an insert (identified by blue-white color selection) were then grown to stationary phase at 37°C in liquid LB media (5 ml) containing 50 μg/ml ampicillin. Plasmid DNA was isolated using the Wizard Miniprep kit (Promega Corp., Madison, WI) and analyzed by restriction analysis with EcoRI for an insert of the correct length. Plasmid DNA (3–5 μg) was sequenced directly with a Sequenase 2.0 kit (USB Biologicals, Cleveland, OH) following the manufacturer's alkaline denaturation protocol. Each nucleotide sequence was determined from at least two independent bacterial colonies.

Adoptive transfer experiments. These experiments were conducted essentially as described by Vlahakos et al. (19) and Tsao et al. (27). Briefly, 10 d after pristane priming, hybridoma cells (∼ 10^7) from 4B2 (IgG2a), 9F11 (IgG2b), 11F8 (IgG3), and 1F/12 (36), an IgG2a secreting control (obtained from the American Type Culture Collection, Rockville, MD), were injected into the peritoneal cavity of 6-wk-old (AKR × DBA/2)F_1 mice (three mice per cell line). Levels of proteinuria, hematuria, and anti-DNA activity were measured before adoptive transfer and before death. Proteinuria was measured using a Chemstrip 6 (Boehringer Mannheim Biochemicals, Indianapolis, IN), and hematuria was quantified as previously described (37, 38). At the onset of visible ascites (∼ 10 d), serum and ascites samples were assayed for anti-DNA activity and total IgG concentration by ELISA as previously described (35). At this point the mice tested positive for anti-DNA in both serum and ascites and were killed by anesthesia. Kidney alterations were assessed by light microscopy using formalin-fixed paraffin-embedded tissue with hematoxylin and eosin, periodic acid-Schiff, and trichrome stains (37, 38). Immune-complex deposition in the kidneys was evaluated by direct immunofluorescence using frozen sections stained with FITC-conjugated goat anti–mouse IgG and C3 (Cappel-Organon Teknika, Durham, NC) as well as by electron microscopy (37, 38). The kidney specimens were read by a pathologist (K.J. Johnson) with no prior knowledge of the pathogenicity of the various antibody clones. The kidneys were evaluated using standard procedures used for assessing biopsies of human lupus patients; i.e., the World Health Organization (WHO) classification as well as the semiquantitative lupus activity and chronicity index (39, 40).

In vitro binding assays. Binding of mAbs to protein and DNA antigens was assessed in two sets of experiments as described by Ohnishi et al. (24). First, Immulon II microtiter plates were coated with either histone (Boehringer Mannheim), collagen type IV (Fluka, Ronkonkoma, NY), fibronectin (Fluka), or laminin (ICN, Costa Mesa, CA) (100 μl of 5 μg/ml solutions in PBS) and blocked with PBS containing 3% BSA (14, 17, 18, 34). mAbs 9F11 and 11F8 (50 ng) were preincubated with either poly(dT) (50 ng), native calf thymus dsDNA (ctDNA), or heat-denatured ctDNA (50 ng) in PBS at 25°C for 1 h followed by 4°C for 18 h. These complexes were added to wells coated with the proteins described above and incubated at 25°C for 2 h. After washing the wells with PBS containing 0.1% Tween-20, bound mAb was detected with alkaline phosphatase–conjugated goat anti–mouse Ig followed by addition of *p*-nitrophenyl phosphate substrate (35). In the second set of experiments, preformed protein–DNA complexes were coated onto microtiter plates before addition of anti-DNA mAbs. Briefly, DNA (50 ng of either poly[dT], native ctDNA, or heat-denatured ctDNA) was preincubated with either histone, laminin, or collagen (50 ng) at 4°C for 18 h and then coated onto microtiter plates. mAbs 9F11 and 11F8 (50 μl of 1 μg/ml solutions in PBS containing 1% BSA and 0.05% Tween-20; PBT) were added to the appropriate wells and incubated at 25°C for 2 h. Bound anti-DNA was detected as described above.

Binding of mAb–DNA/histone complexes to collagen IV, laminin, fibronectin, and heparan sulfate (Fluka) was performed as described by Ohnishi et al. (24). Briefly, 9F11 or 11F8 (50 ng) were preincubated with histone (100 ng) and DNA (100 ng of either poly[dT], native ctDNA, or heat-denatured ctDNA) in PBS at 25°C for 1 h and then at 4°C for 18 h. These complexes were then transferred to appropriate wells precoated with either collagen IV, laminin, fibronectin, (100 μl of 10 μg/ml solutions of each in PBS) or heparan sulfate (100 μl of a 25 μg/ml solution in PBS). After incubating at 25°C for 2 h, the wells were washed, and bound anti-DNA was detected as described above.

mAbs 9F11 and 11F8 were examined for their ability to bind glomerular antigens present in isolated kidney. Briefly, 3-μm thick cryostat sections of naive AKR mouse kidney were mounted on glass slides as previously described (37, 38). One group of kidney sections

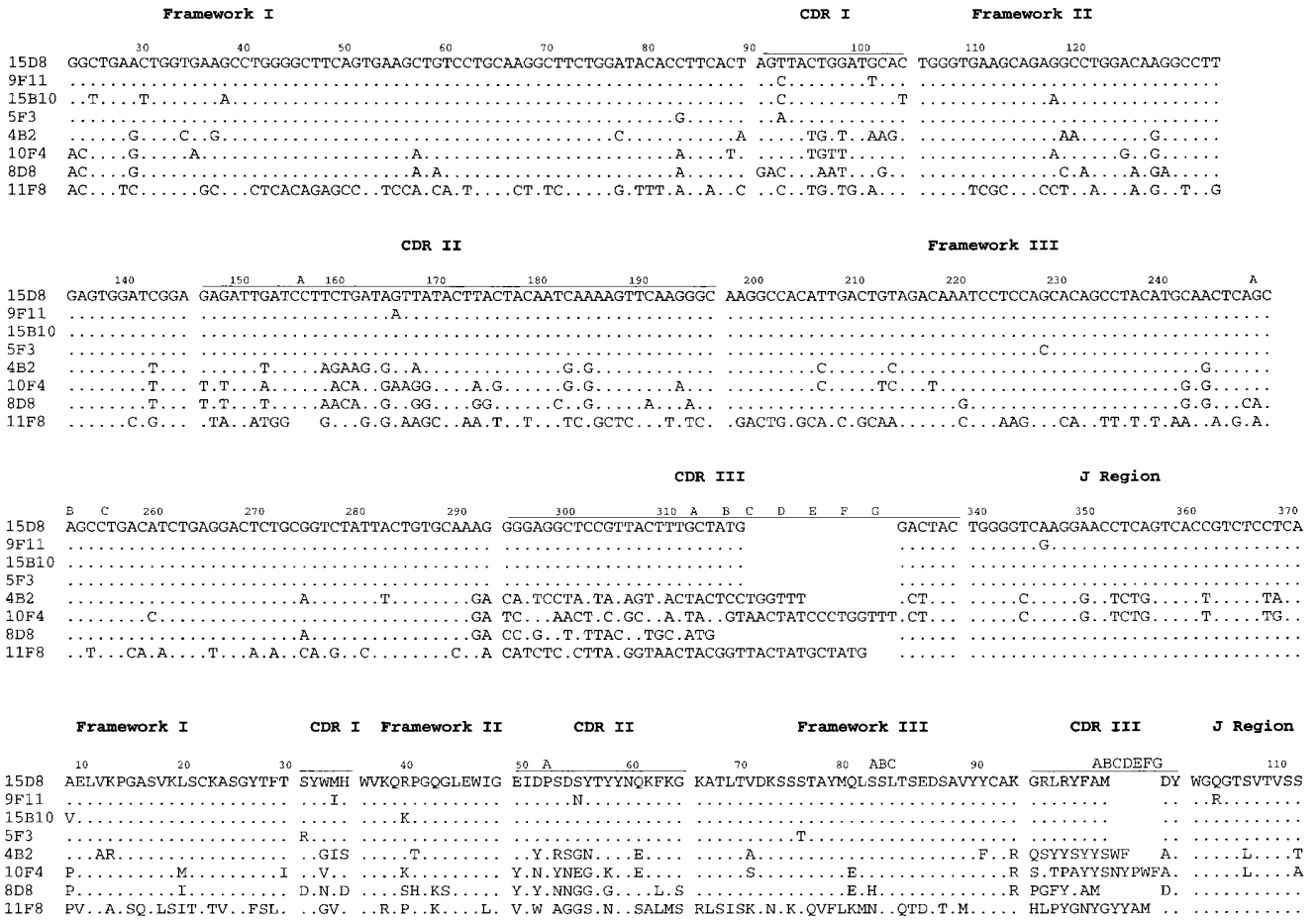

Figure 1. Anti-DNA V_H chain nucleotide and deduced amino acid sequences. Sequences were aligned using the Sequence Analysis WorkBench (UAB Research Foundation, Birmingham, AL). Nomenclature and numbering are according to Kabat et al. (45). Sequences begin after the V_H primer at position 24 in codon 8. Identities are indicated by (.). These sequence data are available from GenBank under accession numbers U28337, U28338, U28339, U28340, U28341, U28342, U28343, and U28344, respectively.

was then treated with DNase I (Sigma, Chemical Co., St. Louis, MO) by immersing the slides in buffer (50 ml) containing 20 mM Tris-Cl, pH 8, 10 mM $MgCl_2$, 100 μg/ml DNase I at 37°C for 1 h (41). After washing with PBS, either normal mouse IgG, 9F11, or 11F8 (100 μl of 10 μg/ml solutions in PBS) were added to kidney slices and incubated at 25°C for 1 h. Bound antibody was detected as described previously (37, 38). In a second group of experiments, the antibody of interest was preincubated with dT_{21} (0.8 μM, 1 h, 25°C) before incubating with the kidney section.

The binding of mAbs 4B2, 9F11, and 11F8 to mouse complement C3 was assessed following the protocol of Ohnishi et al. (24). Briefly, heat-denatured calf thymus DNA (100 μl of a 10 μg/ml solution in TBS) was coated onto Immulon II microtiter plates at 25°C for 18 h. After blocking with PBS containing 3% BSA, purified mAbs (50 μl of a 1 μg/ml solution in PBT) were added to the appropriate wells and incubated at 25°C for 1 h. After washing, freshly pooled BALB/c serum diluted 1:25 in veronal-buffered saline (either with or without preheating at 60°C for 30 min) was added and incubated at 37°C for 1 h (42, 43). Bound complement was visualized by addition of anti-mouse C3 peroxidase conjugate (Cappel) followed by 2,-2′-azino-di-[3-ethylbenzthiazoline sulfonate] substrate (Boehringer Mannheim). A second group of experiments was performed as described above except that the diluted serum was incubated with mAbs that were precoated onto microtiter plates (50 μl of 1 μg/ml solutions in PBS) at 25°C for 18 h and blocked with PBS containing 3% BSA.

Serum and ascites were tested for the presence of antiidiotype antibodies before and after adoptive transfer of 11F8 hybridomas by ELISA. Briefly, the F(ab) of 11F8 was prepared and purified according to the procedure of Swanson et al. (35). Microtiter plates were coated with 11F8 F(ab) (50 μl of a 1 μg/ml solution in PBS) at 25°C for 18 h and blocked with PBS containing 3% BSA. Serum and ascites diluted 1:100 in PBT were added and incubated for 2 h at 25°C. Bound IgG was detected using anti–mouse Fc alkaline phosphatase conjugate (Cappel) followed by addition of *p*-nitrophenyl phosphate substrate (Sigma Chemical Co.).

Cryoglobulin assays. Cryoglobulin activity was assessed in two experiments (44). In the first, serum was obtained from a normal BALB/c mouse or a BALB/c producing 11F8 in ascites. After clotting at 37°C for 2 h, the serum from both mice was split into two aliquots (100 μl each) and maintained at 4°C for 7 d. Both samples were visually inspected daily for the presence of a precipitate. At no time during the 7 d was a precipitate detected as judged by visual inspection of the samples. After 2 d, one serum sample from each of the mice was centrifuged at 3,000 rpm at 4°C for 10 min. After decanting the supernatant, a small amount of a viscous gel-like residue was observed in both samples which did not redissolve after one washing with cold PBS. Both residues were dissolved in SDS loading buffer (5 μl), boiled for 5 min, and aliquots (1 μl) were electrophoresed on denaturing polyacrylamide gels with a continuous gradient of polyacrylamide (10–15%) and stained with Coomassie blue according to the manufacturer's protocol (PhastGel Gradient 10-15%; Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). This procedure was repeated for the aliquot stored for 7 d at 4°C. Control electrophoresis experiments using normal mouse IgG and affinity-purified 11F8 show

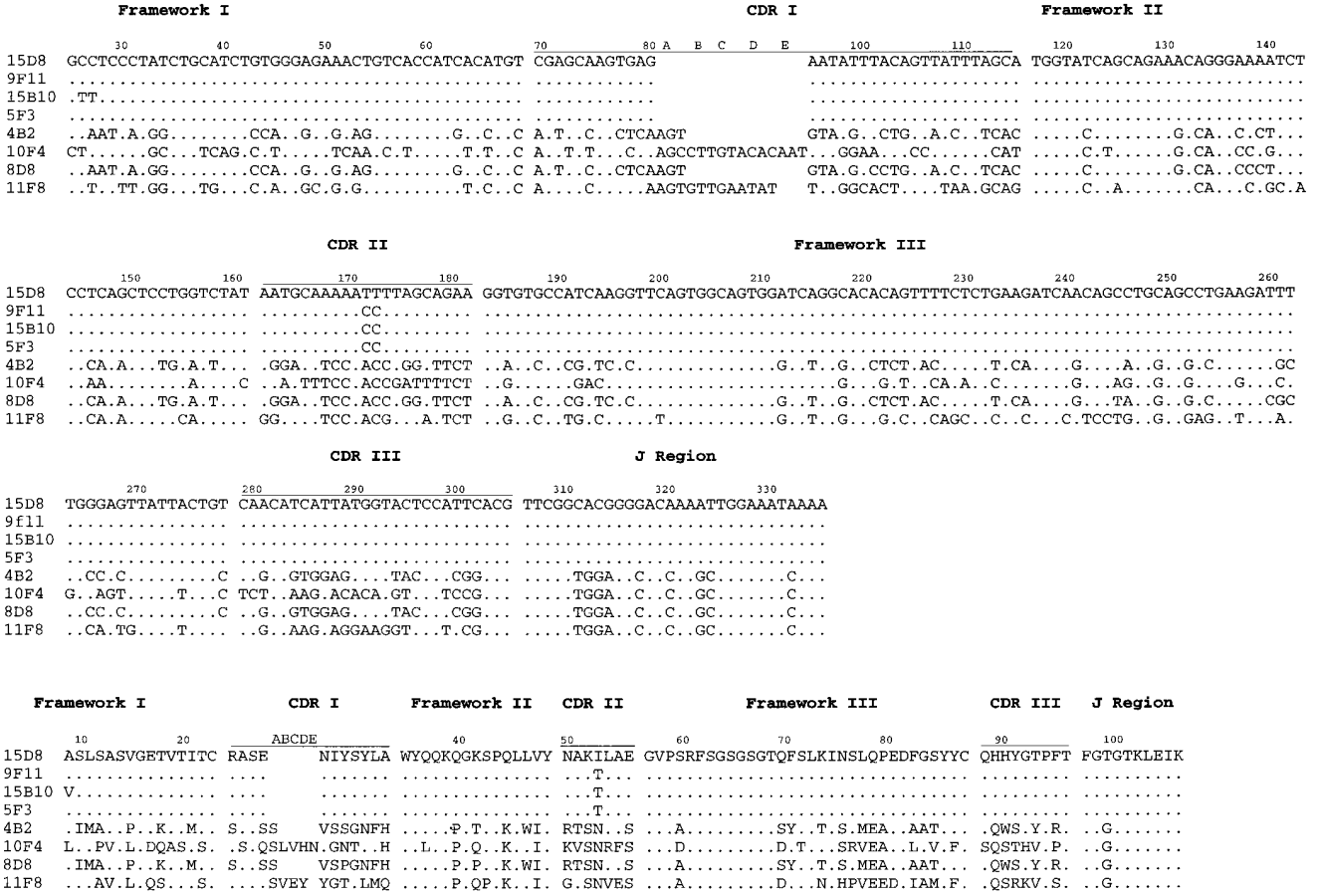

Figure 2. Anti-DNA V_κ chain nucleotide and deduced amino acid sequences. Sequences are presented according to the legend of Fig. 1. Sequences begin after the V_κ primer at position 25 in codon 9. These sequence data are available from GenBank under accession numbers U28345, U28346, U28347, U28348, U28349, U28350, U28351, and U28352, respectively.

that the detection limit for these gels is $\sim$ 30 ng of antibody per 1 μl which agrees with detection limits quoted by the manufacturer.

The second cryoglobulin assay was performed as the first, except that purified 11F8 and normal mouse IgG (1 ml of 1 mg/ml solutions in PBS) was used in place of serum. As with the serum samples, visible precipitates did not form over the 7-d incubation period at 4°C. In addition, centrifugation did not afford a visible precipitate. Because small quantities of material may not be easily observed, the supernatant was decanted, the bottom of the tube was washed with cold PBS, and the wash was electrophoresed as described above.

Results

V gene analysis. The V_H and V_L nucleotide sequences and the corresponding gene families were determined for eight anti-DNA mAbs (Figs. 1 and 2, and Table I, respectively). Clones 9F11, 15B10, 15D8, and a low avidity mAb, 5F3, are > 99% identical to each other and possess identical V_HDJ_H and $V_\kappa J_\kappa$ junctional regions which indicates that they are clonally related (53, 54). The remaining four mAbs are all single isolates. Seven of the V_H genes that encode our mAbs are derived from the J558 family while one clone uses V_HQ52. These findings are consistent with previous work showing that the J558 gene family accounts for the majority of anti-DNA V_H genes (21, 22). Radic and Weigert have described ten homology subgroups that account for most of the V_HJ558 encoded anti-DNA (21). mAbs 8D8 and 10F4 belong to subgroups 7 and 8, respectively, while the other six clones are sufficiently different to make assignment ambiguous. Similarly, in addition to the clonally related mAbs, 4B2 and 10F4 are encoded by genes that are members of the same V_HJ558 subfamily defined by Rajewsky et al. (48).

Four different V_κ genes encode the light chains of the anti-DNA in our panel. Of the mAbs whose V_HJ558 subgroup/subfamily could be assigned, V_HJ558-V_κ pairings shown previously to be recurrent are not observed (21, 22). Comparison of the gene structures of our anti-DNA to published anti-DNA V_H and V_κ genes reveals that the most closely related sequences are between 93 and 99% homologous, which suggests that this panel of mAbs is representative of anti-DNA commonly expressed in lupus-prone mice. Previous analysis of anti-DNA V genes provides evidence for structural motifs/residues that may be associated with DNA binding. These include, among others, ^{N35}HCDR1, ^{Y100}HCDR3, the sequence YYGS in HCDR3, R in HCDR3, and ^{R96}LCDR3 (particularly in association with $J_\kappa 1$) (21–32). Brief inspection of the deduced amino acid sequences of our mAbs shows that ^{Y100}HCDR3 is present in 10F4 and 4B2, the group of clonally related mAbs contains R at positions 96 and 98 of HCDR3, and the V_κ genes of mAbs 4B2 and 8D8 encode ^{R96}LCDR3.

Because most of the germline V genes in MRL-*lpr* mice are

Table I. Anti-DNA Gene Usage

Clone	ssDNA Binding*	dsDNA Binding*	Isotype	V_H‡ family	J558§ Subgroup	J588‖ Subfamily	D_H¶	J_H**	V_κ‡‡	V_κ§§ Subgroup	Jk‖‖
9F11	+++	NC	IgG2b	J558	NA	V3	Q52¶¶, FL16.2	4	12, 13	NA	4
15B10	+++	NC	IgG2b	J558	NA	V3	Q52¶¶, FL16.2	4	12, 13	NA	4
15D8	+++	NC	IgG2b	J558	NA	V3	Q52¶¶, FL16.2	4	12, 13	NA	4
5F3	+	NC	IgG2a	J558	NA	V3	Q52¶¶, FL16.2	4	12, 13	NA	4
4B2	++	+++	IgG2a	J558	NA	V186.2	SP 2.7	3	5	5	1
8D8	++	NC	IgG2a	J558	7	205.12	Q52r	4	5	5	1
10F4	++	+	IgG2a	J558	8	V186.2	SP 2.7	3	1	1A	1
11F8	+++	NC	IgG3	Q52	—	—	SP 2.5, 2.7	4	21	***	1

A brief summary of mAb binding affinity/specificity has been included in this table for comparison. *ssDNA refers to oligo(dT) and dsDNA is native calf thymus DNA. Equilibrium binding constants for oligo(dT) were measured by gel shift or fluorescence quenching (35). Binding of dsDNA was conducted by both direct and competition ELISA. +++, strong binding; ++, moderate affinity; +, weak binding; NC, no complex is formed. ‡Designation based on > 80% homology to members within V_H gene family described by Brodeur and Riblet, and Kofler et al. (46–47). §Assignment according to Radic and Weigert (21). Subgroup designation requires that no more than four amino acids differ from the derived consensus subgroup amino acid sequence in HCDR1 and HCDR2. NA, not assignable. ‖Designation based on highest nucleotide identity with V_HJ558 subfamily members described by Gu et al.(48) ¶D gene assignments are based on homology to sequences reported by Kurosawa and Tonegawa (49). D genes followed by (r) are inverted. ¶¶D gene assignment tentative based on limited overlap with known germline D gene nucleotide sequences (46). **Classification according to Sakano et al. (50). ‡‡Designation according to Potter et al. (51). §§Subgroups defined by Radic and Weigert (21). NA, not assignable. ***assignment ambiguous. ‖‖Classification according to Sakano et al. (52).

not known, it is not possible to precisely analyze our mAbs with respect to somatic mutations, except for those mutations involving the J minigenes whose germline sequences are known (45, 50, 52). Further analysis of the nucleotide/amino acid sequences of these antibodies does, however, illuminate several interesting features. For example, in the heavy chain of 4B2, R occurs at position 53 in HCDR2 and R is also found at this position in other anti-DNA that bind dsDNA like 3H9 (54). MAb 10F4 also binds dsDNA, albeit weakly, and this mAb does not possess ^{R53}HCDR2. Collectively, these findings support the hypothesis of Weigert and co-workers proposing that ^{R53}HCDR2 is important for (high affinity) recognition of dsDNA (55). The HCDR3 of 4B2 is apparently constructed from a $D_{SP2.7}$ gene element with short N sequences at the 5′ and 3′ ends. The 4B2 J_H3 gene contains a substitution at codon 103 coding for A in place of T. ^{R96}LCDR3 is found in the light chain of 4B2 and Marion and co-workers have suggested that ^{R96}LCDR3 is correlated with dsDNA specificity (22). While 4B2 binds dsDNA, the absence of dsDNA reactivity in clone 8D8, which also possesses ^{R96}LCDR3, suggests this correlation is not strict. Furthermore, 10F4 does not contain ^{R96}LCDR3 and does bind dsDNA.

mAbs 9F11, 15B10, 15D8, and 5F3 are clonally related, however, their genealogy cannot be determined due to the lack of sufficient parallel and unique somatic mutations that define evolutionary branchpoints (23, 53, 54). 9F11 differs from the other antibodies at codons 34 and 55, substituting I for M in HCDR1 and N for S in HCDR2, respectively. Construction of HCDR3 appears to involve fusion of D_{Q52} and $D_{SP16.2}$ genes. The codons formed from the D_H-D_H junction and the N addition 3′ of the $D_{SP16.2}$ segment encode R at positions 96 and 98 of HCDR3. Both of these mechanisms have been described previously to explain the origin of R in HCDR3 of anti-DNA (56, 57). The J_H4 gene of these four antibodies contain a TAT to TTT conversion resulting in a conservative substitution in HCDR3 of Y100F. MAb 9F11 possesses a CAA to CGA mutation at position 105 of the J_H gene replacing Q for R. In the V_κ gene, clones 9F11 and 5F3 are identical, whereas 15B10 differs, substituting V for A in FR1, and 15D8 loses a hydrogen bond donor/acceptor in a T to I mutation in LCDR2. All four mAbs in this group use a $J_\kappa4$ element that contains a conservative S to T substitution at position 100.

The observation that 5F3 possesses only low avidity to ssDNA whereas 9F11, 15B10, and 15D8 bind ssDNA with relatively high (monovalent) affinity (35) suggests that a mutation in 5F3 strongly interferes with ligand recognition. Aside from a conservative S76T mutation, the only other mutation unique to 5F3 is S31R in HCDR1. Although mutations to R are generally thought to improve affinity for DNA (55), S31R may disrupt the geometry of the antigen combining site by altering the conformation of the HCDR1, a hypothesis that has recently been proposed by Ibrahim et al. (58). 5F3 is not the only example of an anti-DNA mAb in which lower affinity results after mutation to R. For example, Weigert et al. has demonstrated that a K64R mutation in HCDR2 of 3H9 completely abolishes DNA binding activity (55).

Pathogenicity of three anti-DNA mAbs. Three representative mAbs from our panel, 4B2 (IgG2a, κ), 11F8 (IgG3, κ), and 9F11 (IgG2b, κ), were selected for further study. These mAbs were chosen based on their binding specificity, gene usage, and physical properties that may be associated with pathogenicity. Clone 4B2 has several traits that pathogenic anti-DNA are believed to possess (e.g., an IgG class and reactivity towards dsDNA; 6). mAb 11F8, an IgG3, was chosen because production of IgG3 antibodies has been implicated in the development of glomerulonephritis in MRL-*lpr* mice (59, 60). The last mAb, 9F11, represents a relatively high affinity anti-ssDNA that belongs to a group of clonally related antibodies. The binding mode and affinity of 9F11 for oligo(dT) is similar to that for 11F8 (35) which provides an opportunity to test the relationship between the mode of ligand binding, gene structure, and pathogenicity.

Hybridoma cells that produce mAbs 4B2, 9F11, and 11F8, as well as an irrelevant IgG2a mAb (1F/12) were administered

Table II. Antigen Binding, Complement Fixation, and Clinical Effects of 4B2, 9F11, and 11F8

Hybridoma	Isotype	Serum anti-DNA preinjection*‡	Serum anti-DNA post-transfer*‡	Serum IgG conc.‡§	Ascites IgG conc.‡§	C′ fixation‡‖	Proteinuria	Hematuria	Kidney deposition‡¶	Severity‡**
		mean OD_{405}	*mean OD_{405}*	*mg/ml*	*mg/ml*		*mg/dl*	*erythrocyte per μl*		
1F/12	IgG2a	0.108	0.151	20.9	~ 50	NM	100	10–50	none	1
4B2	IgG2a	0.113	1.65	5	7.5	2.25 (0.117)	100	50–250	mes	2
9F11	IgG2b	0.165	2.31	10.4	9.1	2.36 (0.106)	100–500	50–250	mes	2+
11F8	IgG3	0.141	1.34	7.4	5.6	1.4 (0.116)	100–500	50–250	mes/subendo	4+

*Serum or ascites were diluted 1:100 and measured for binding to heat-denatured ctDNA by ELISA (see Methods). The values presented are the mean of triplicate measurements. The background using BALB/c sera diluted 1:100 did not exceed 0.2 A.U. ‡The data presented are for one mouse. However, within each group of mice for each of the four mAbs, similar results were obtained. §The concentration of IgG was determined using a commercially available ELISA kit (Boehringer Mannheim). Note that the amount of circulating IgG is not correlated to the induction of nephritis since mice administered with 1F/12 have larger quantities of circulating mAb than those inoculated with 11F8 hybridoma cells. ‖Complement fixation as assayed through binding of murine C′3 by an ELISA. Presented are means of triplicate measurements taken at 405 nm after 15 min. The values obtained using heat-denatured samples are shown in parentheses. NM, not measured. ¶Mes, mesangial; subendo, subendothelial. Assessed by direct immunofluorescence and electron microscopy according to the WHO classification (39). **Severity (0–4+) according to the semiquantitative lupus activity and chronicity index (40). conc., concentration.

to 6-wk-old pristane primed nonautoimmune (AKR × DBA/2)F_1 mice to determine which, if any, of these mAbs induce nephritis in vivo. This type of adoptive transfer protocol is an established method that has been used in several laboratories to assess the pathogenicity of anti-DNA mAbs (6, 19, 20, 24–29, 33). We chose this approach over intravenous injection of purified mAbs for these initial experiments because it does not require large amounts of purified antibody or extensive pharmacokinetic evaluation. Moreover, adoptive transfer of hybridoma cells is reported to provide levels of circulating Ig that resem-

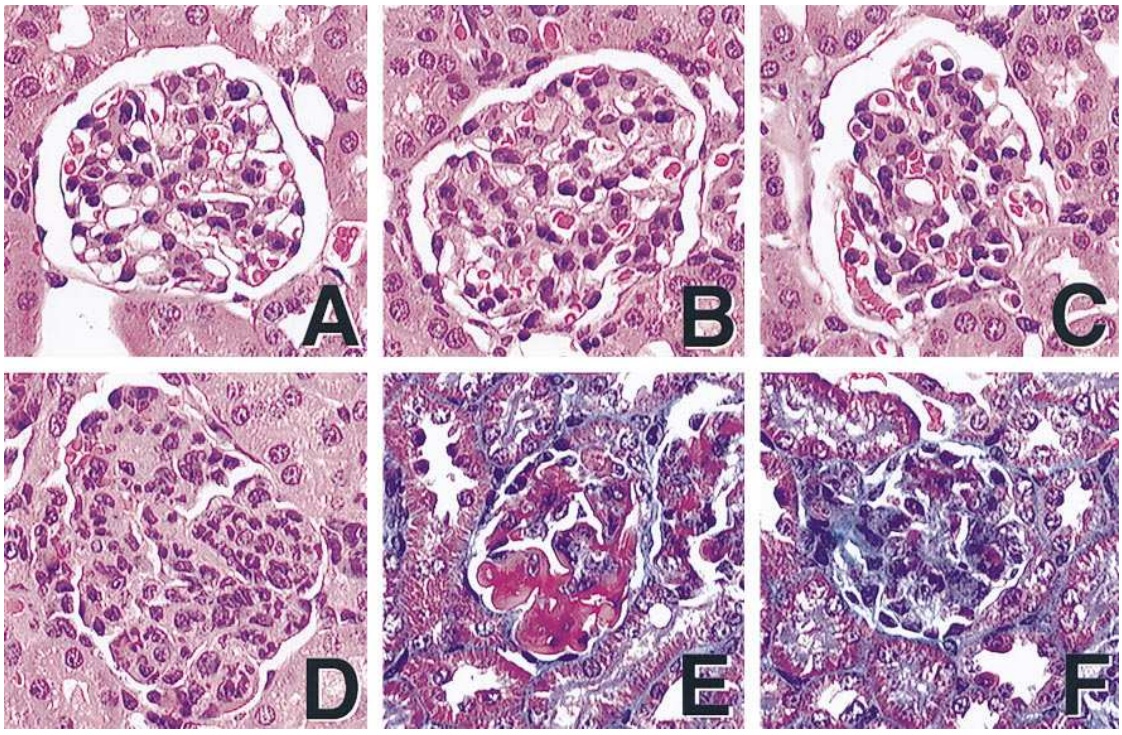

Figure 3. Representative histologic appearance of glomerular alterations following administration of 1F/12, 4B2, 9F11, and 11F8 hybridomas (all panels are ×400). (*A*) Animals receiving 1F/12 have normal glomeruli by light microscopy. Animals receiving either (*B*) anti-dsDNA 4B2 or (*C*) anti-ssDNA 9F11 possess only mild mesangial proliferation with a slight expansion of the mesangial matrix with no significant neutrophilic infiltration. Animals receiving 11F8 hybridoma cells have a severe diffuse proliferative glomerulonephritis with marked hypercellularity including (*D*) large numbers of neutrophils, (*E*) widespread immune deposits including many large subendothelial wire-loop lesions, and (*F*) glomerular sclerosis. *A–D* are stained with hematoxylin and eosin and *E–F* are stained with Masson trichrome. Similar data to those given in this Figure and in Figs. 4 and 5 have been obtained from three additional sets of adoptive transfer experiments with 11F8 (15 additional mice total) which indicates that generation of the severe disease with 11F8 is reproducible.

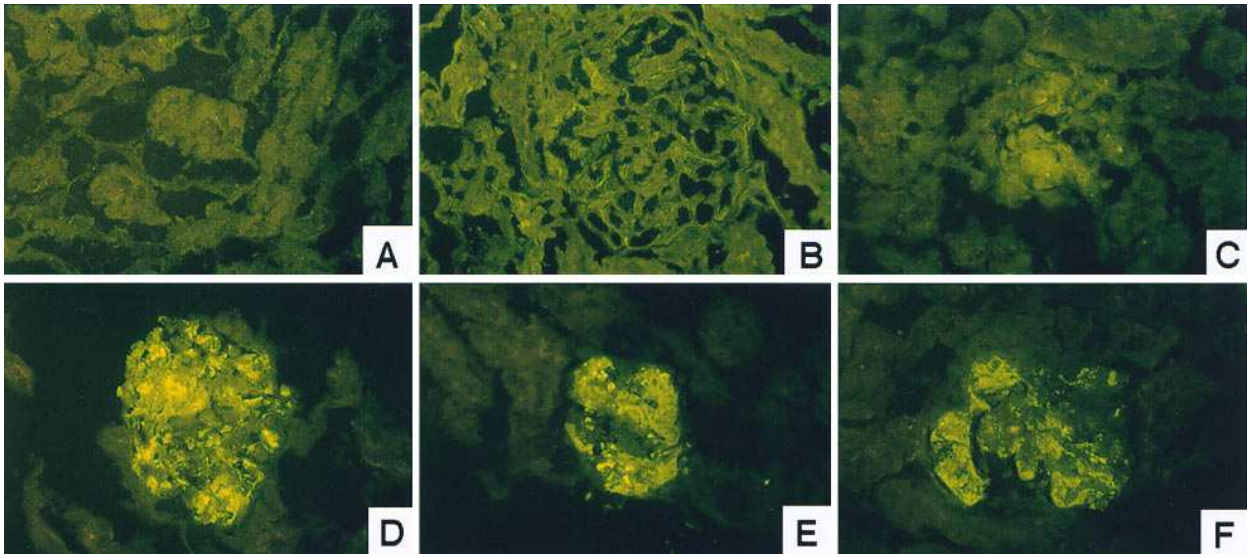

Figure 4. Representative direct immunofluorescence of kidney sections from mice that received 1F/12, 4B2, 9F11, or 11F8 hybridomas (all panels are ×400). (*A*) The control 1F/12 animals show little immune deposition in the glomeruli (∼ 1–2). Adoptive transfer of either 4B2 (*B*) or 9F11 (*C*) hybridoma cells induces minimal immune deposition in the glomeruli. Hybridoma 11F8 show 4+ immune deposition in glomeruli (*D–F*). Most of the immune deposition is present in the mesangial regions, however, focal linear-granular capillary wall immune deposits are also present (*F*).

ble conditions associated with active disease (19). All animals produce ascites within ∼ 10 d and develop proteinuria and hematuria, including to some extent, the control animals (Table II). Levels of anti-DNA in serum and ascites are very similar (data not shown), although the total level of IgG produced varies among groups of mice.

Light microscopy of kidney sections from each of the mice treated with 11F8 show a prominent diffuse proliferative type of glomerulonephritis (WHO class IV) with marked hypercellularity, intracapillary necrosis, and infiltration by polymorphonuclear leukocytes (Fig. 3). Distinctive "wire-loop" lesions are present in many glomeruli, and early chronicity changes are present with scarred glomeruli. Immunofluorescence and electron microscopy studies show intense (4+) diffuse mesangial and subendothelial immune deposits with ultrastructural evidence of widespread subendothelial dense deposits that correlates with the wire-loop lesions seen by light microscopy. (Figs. 4 and 5). Taken together, these observations are indicative of a diffuse proliferative type of glomerulonephritis that resembles very closely the severe diffuse proliferative glomerulonephritis seen in patients with SLE (39). By contrast, the kidney sections from the animals treated with hybridoma cells producing mAbs 4B2 and 9F11 are not remarkably different from the negative control. The glomeruli from each of the animals treated with 4B2 and 9F11 show mild mesangial proliferation by light microscopy (Fig. 3) and weak (1–2+) mesangial immune deposition by immunofluorescence (Fig. 4). Moreover, adoptive transfer of these mAbs does not produce scarring or show evidence of chronicity. Applying the renal biopsy scoring system used for lupus nephritis, each of the 11F8-treated animals have an average activity index of 14/24 versus 5/24 for the animals given the 4B2 and 9F11 antibodies (40). Scores for the control mice are each about 2/24.

Potential mechanisms of pathogenicity. It is striking that anti-DNA mAbs apparently possessing similar affinity, specificity, and mode of binding in vitro (35) can differ so remarkably in their effects in vivo. One explanation for these observations is that 11F8 directly binds a component of the GBM that 4B2 and 9F11 do not recognize, or do so with low affinity (11–20). However, none of the mAbs binds either to purified antigens found normally within the GBM or to kidney sections treated with DNase I (vide infra). Yet it is possible that 11F8–DNA complexes could either selectively deposit within glomeruli or 11F8 might recognize epitopes presented in the context of protein–DNA complexes localized within the GBM (16–18, 24). To test the first of these hypotheses, we examined whether 11F8 and 9F11 complexed to either poly(dT), ssDNA (i.e., heat-denatured ctDNA), or dsDNA bind immobilized components of the GBM: histone, laminin, collagen IV, and fibronectin (Table III). mAbs 11F8 and 9F11 both form complexes with poly(dT) and ssDNA that bind strongly to histone. Complexes with poly(dT) bind weakly to laminin and collagen IV, but not to fibronectin, whereas complexes with ssDNA do not bind either laminin, collagen IV, or fibronectin. Since 11F8 and 9F11 do not bind dsDNA, neither mAb preincubated with dsDNA binds to any of the proteins tested. To examine the second hypothesis, we measured the affinity of both mAbs for DNA precomplexed to either histone, collagen IV, laminin, or fibronectin. In these experiments, we find that 11F8 and 9F11 both bind to protein complexes containing poly(dT) and ssDNA. The affinity of both mAbs for protein–DNA complexes measured in these assays is comparable to that observed in the experiments described above. Collectively, these results suggest that 11F8 and 9F11 recognize protein–DNA complexes which may serve as planted antigens in vivo (16–18, 24). However, the reactivity of both mAbs in each of the ELISA experiments is comparable and therefore cannot provide a basis to account for the severity of the tissue injury caused by 11F8 relative to that observed for 9F11.

To explore the possibility that 11F8–histone–DNA immune complexes localize to the glomeruli via interactions between histone and the GBM, we investigated whether ternary

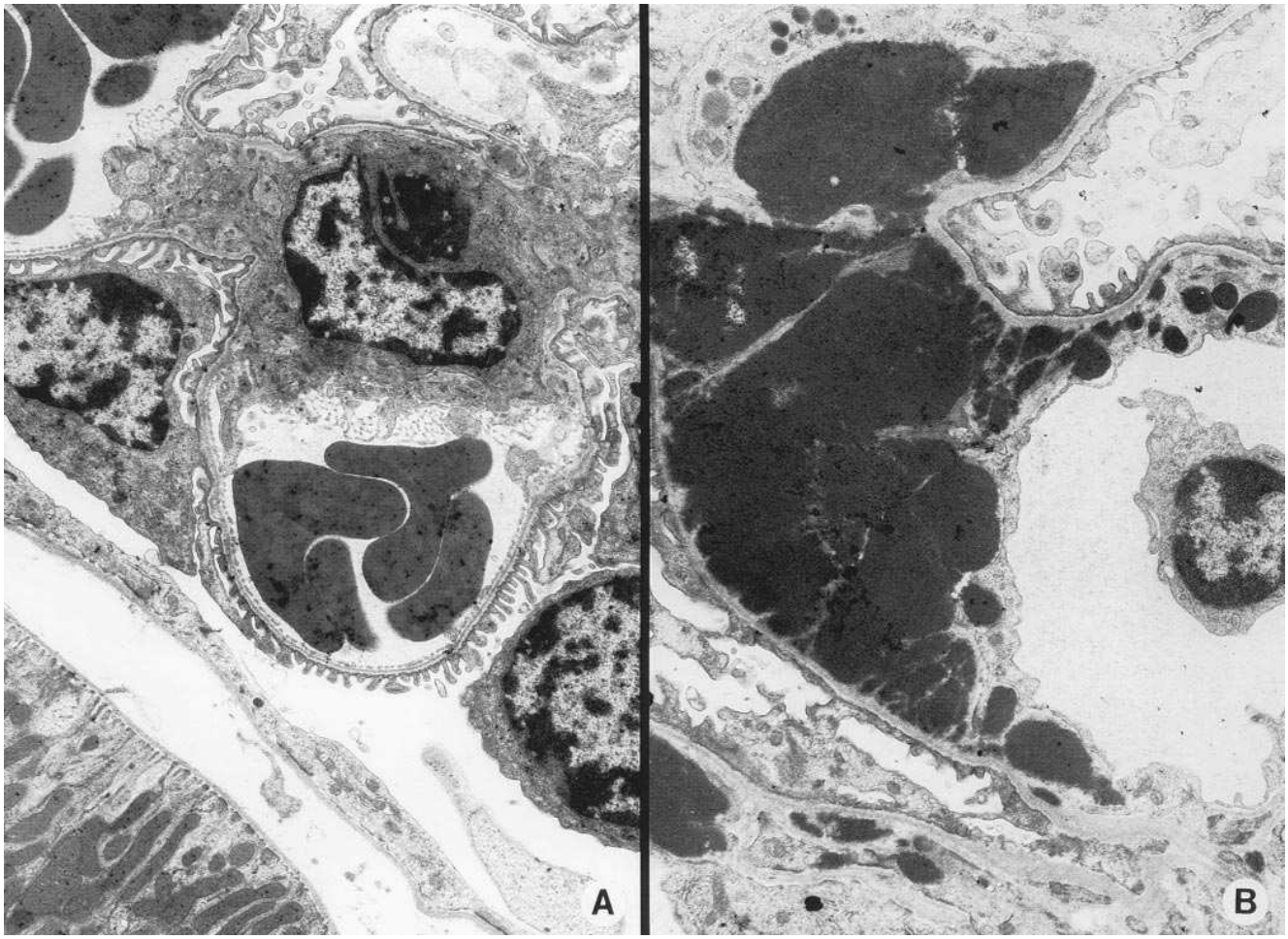

Figure 5. Representative electron micrographs of glomerular alterations from mice inoculated with 1F/12 and 11F8 hybridoma cells (*A*, ×7,300; *B,* ×8,650). (*A*) Glomerular ultrastructure after adoptive transfer of 1F/12. The glomerulus appears normal. (*B*) The typical ultrastructural appearance of glomeruli from animals inoculated 11F8 hybridoma cells. Note the large numbers of electron dense deposits in the glomeruli present in both the mesangial and subendothelial regions.

Table III. In Vitro ELISA Experiments

Complex	Histone	Laminin	Collagen IV	Fibronectin	Heparan sulfate
9F11•dsDNA	—	—	—	—	NM
11F8•dsDNA	—	—	—	—	NM
9F11•ssDNA	+++	—	—	—	NM
11F8•ssDNA	+++	—	—	—	NM
9F11•poly(dT)	+++	++	+	—	NM
11F8•poly(dT)	+++	++	+	—	NM
9F11•dsDNA•histone	NA	—	—	—	—
11F8•dsDNA•histone	NA	—	—	—	—
9F11•ssDNA•histone	NA	+	+	—	—
11F8•ssDNA•histone	NA	+	+	—	—
9F11•poly(dT)•histone	NA	++	+	—	—
11F8•poly(dT)•histone	NA	++	+	—	—

Data are the mean of duplicate measurements. The background reactivity to wells containing no antigen did not exceed 0.08 A.U. (—), < 2× background; (+), 2–5× background; (++), 5–10× background; (+++), > 10× background. NA, not applicable. NM, not measured.

complexes consisting of histone, mAb, and DNA (either poly[dT], ssDNA, or dsDNA) bind to immobilized heparan sulfate, collagen IV, laminin, or fibronectin (Table III) (16, 17, 24). 11F8 and 9F11 both form complexes with histone and either ssDNA or poly(dT) that bind to laminin and collagen IV, but not to heparan sulfate or fibronectin. Importantly, the levels of antibody binding to laminin and collagen IV are somewhat higher in the presence of both histone and DNA than with DNA alone. These results suggest that binding of ternary immune complexes to the GBM may also provide a mechanism for the glomerular localization of anti-DNA (16, 17, 24). Again, however, the reactivity of 11F8 and 9F11 toward protein–DNA and histone–DNA complexed to extracellular matrix components is similar and cannot provide a means to resolve the observed differences in pathogenicity between the two mAbs.

Because there are antigens (and perhaps conformational epitopes) in vivo that are not represented in our in vitro experiments, we examined the binding of normal mouse IgG (nIgG), 11F8, and 9F11 to isolated naive kidney sections by indirect immunofluorescence (17, 24). As expected, normal mouse IgG does not bind to either unmanipulated or DNase I–treated kidney sections. By contrast, 11F8 binds to unmanipulated kidney sections (3+ through 4 localized to the glomeruli relative to a background of 1 through 2+; others have observed background immune-complex deposition in normal mice, see references 30, 37, 38). This binding is decreased to background levels either by pretreatment of the kidney sections with DNase I (17, 24, 33) or by preincubation of 11F8 with dT_{21}, which binds with an apparent equilibrium dissociation constant in the low nanomolar range (Fig. 6). While it is possible that mAb 9F11 also binds to naive kidney sections, the fluorescence intensity is not significantly above background. These experiments indicate that 11F8 specifically recognizes DNA epitopes adherent to glomeruli in support of the planted antigen hypothesis of glomerular localization (16, 17, 24, 33). Although 11F8 stains naive kidney tissue more strongly than 9F11 in these in vitro studies, the difference is not as large as that observed in the immunofluorescence experiments using kidney sections from animals treated with the different mAbs (Fig. 4). However, it is possible that in vivo, repetitive cycles of binding, tissue damage, and release of antigen at the site of injury lead to binding of more antibody, thereby increasing the fluorescence intensity relative to that observed in the in vitro binding experiments. Alternatively, the increased binding may reflect the fact that in the adoptive transfer experiments high concentrations of 11F8 are present for 10 d whereas in the indirect immunofluorescence experiments, 11F8 is incubated with kidney sections for a much shorter period of time.

Three sets of experiments were performed to determine if factors unrelated to antigen recognition are responsible for the severe tissue injury induced by adoptive transfer of 11F8. First, we measured the ability of 4B2, 9F11, and 11F8 to fix complement. All three mAbs fix complement to the same extent. This observation is consistent with data obtained in other laboratories and supports the proposal that pathogenic and nonpathogenic anti-DNA cannot be distinguished by their ability to fix complement in vitro (24). While it is possible that IgG3 mAbs fix complement to a larger extent in vivo thereby providing a basis to explain the differences between 11F8, 9F11, and 4B2, not all IgG3 mAbs from MRL-*lpr* mice are pathogenic (19, 59–61). Second, we explored the possibility that adoptive transfer of 11F8 initiates an antiidiotype cascade that itself mediates the pathogenicity associated with 11F8 (6, 30, 62, 63). We find no evidence of anti-11F8 antibodies by ELISA (data not shown).

Lastly, we determined if 11F8 is a cryoglobulin since Izui and co-workers have suggested that some IgG3 cryoglobulins (not necessarily anti-DNA) from MRL-*lpr* mice mediate immune-complex formation through Fc-Fc interactions (60, 61). Visible cryoprecipitates were not observed in daily examination of normal mouse serum and serum samples containing 11F8 stored at 4°C for 7 d (44). In addition, purified 11F8 shows no evidence of precipitation even after 2 wk. Centrifugation of both normal mouse serum and serum containing 11F8 affords a gel-like residue that does not redissolve in cold PBS. By contrast, visible precipitates do not form after centrifugation of purified 11F8. Electrophoretic analysis demonstrates that the residue recovered after centrifugation of 11F8 serum does not contain immunoglobulin, but rather it is a mixture of proteins (e.g., albumin) that also precipitates from normal mouse serum (data not shown). By contrast, $<$ 50 ng of

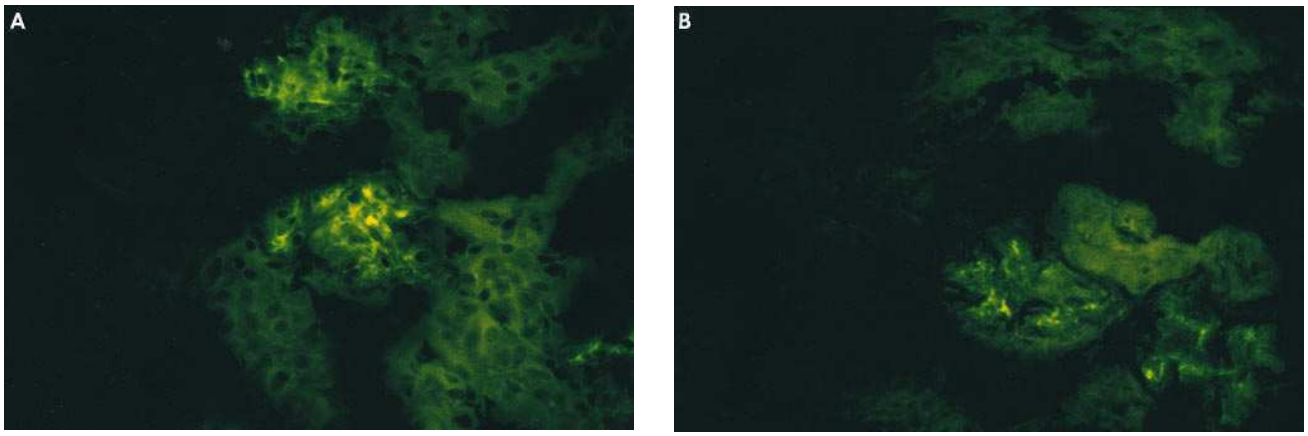

Figure 6. Representative indirect immunofluorescence data for binding of 11F8 to normal kidney tissue. These experiments were conducted as described in Methods. 11F8 incubated with (*A*) normal kidney tissue and (*B*) normal kidney tissue previously treated with DNase I. The fluorescence intensity observed in *B* is similar to that observed for background signal in normal mouse kidney (data not shown). Similar results to that presented in *B* are obtained if 11F8–dT_{21} complexes are incubated with normal kidney sections.

11F8 is recovered after centrifugation of the purified mAb. However, this amount of protein is comparable to that remaining in samples containing the purified normal mouse IgG control and therefore probably represents nonspecific background adsorption of immunoglobulin to the sample tube. Taken together, these results suggest that 11F8 is not a cryoglobulin.

Discussion

Elucidating the features of anti-DNA that are related to their activity both in vitro and in vivo remains a key goal in SLE research (1, 2, 6, 21–23). Although the genes that encode anti-DNA have been extensively studied (21–23), the molecular basis of DNA recognition by anti-DNA (35, 64), and the relationships between anti-DNA–DNA complex formation in vitro and disease pathogenesis in vivo are not fully defined (6). One problem is that few anti-DNA mAbs have been evaluated with respect to their capacity for inducing nephritis, so that attributes distinguishing pathogenic from nonpathogenic anti-DNA have not been defined (6, 11–16, 23–32). To address these issues, we previously generated several anti-DNA mAbs from an MRL-*lpr* mouse and characterized their affinity, specificity, mode, and mechanism of DNA binding (35). Here we describe their genetic origins and the ability of three clones to induce nephritis in vivo.

As previously described, mAbs in our panel are specific for DNA; they do not bind RNA, ribonucleoproteins, phospholipids, proteoglycans, and extracellular matrix components present in the GBM such as collagen IV (35). mAbs 9F11, 15B10, and 15D8 appear to be clonally related which is consistent with biophysical data showing that these anti-DNA share very similar DNA binding properties (35). Although 11F8 and 9F11 also possess similar affinity and specificity for ssDNA homopolymers, both mAbs are encoded by different V genes which themselves have limited nucleotide and amino acid identity with each other (e.g., 57% between the V_H nucleotide sequences and 63% between the V_κ genes). Two other well characterized thymine-specific anti-ssDNA, BV04-01 and HEd10, possess ligand-binding characteristics comparable to 9F11 and 11F8 (65–71) but are encoded by different V genes (e.g., V_H10 and V_HJ606, respectively; 71, 72) that also possess limited homology to the 11F8 and 9F11 V genes. How can DNA specificity and affinity be so similar for these mAbs despite their use of V_H genes that bear relatively low homology to each other? One possibility is that anti-DNA V genes encode a limited repertoire of three-dimensional motifs that form the basis of ssDNA reactivity. This hypothesis is supported by recent crystallographic and molecular modeling studies which indicate that the combining sites of high affinity anti-ssDNA, as a group, are similar and possess steroelectronic complementarity to ssDNA ligands, regardless of the gene families (or mouse strain) from which the mAbs are derived (72, 73).

The V genes used by our mAbs occur in anti-DNA obtained from both autoimmune and nonautoimmune mice. For example, both the 9F11 group of mAbs and clone 11F8 are encoded by V_H genes that are similar to those found in anti-DNA obtained from normal mice immunized with bacterial DNA or protein–DNA complexes, respectively (74, 75). The similarity between autoimmune and immunization-induced anti-DNA has been noted previously and supports the hypothesis that the development of anti-DNA in normal and lupus-prone mice may occur by a common mechanism in which DNA or a protein–DNA complex stimulates B cells to differentiate in a receptor mediated response (74–77). The observation that normal mice immunized with Fus1–DNA complexes, from which mAb bfd89 was derived (75), also develop mild nephritis (76) supports this proposal and suggests that anti-DNA generated by immunization may have some similarity to pathogenic lupus autoantibodies. Moreover, these results suggest that the normal immune repertoire may encode pathogenic anti-DNA.

Pathogenicity: histologic manifestations. The ability of 11F8 to acutely induce a severe glomerulonephritis that resembles the diffuse proliferative form of human lupus nephritis with prominent neutrophil accumulation, wire-loop lesions, and rapid progression to glomerular scarring is unusual when compared to other models of glomerulonephritis in mice, including murine models of lupus. Most models of glomerulonephritis in rodents are typically characterized by mild glomerular alterations with mesangial immune deposits and mesangial proliferative changes observable by light microscopy, particularly when mAbs or other preformed immune-complexes are used to induce the glomerular injury (78). Significant numbers of neutrophils, intracapillary necrosis, and crescents with early fibrosis are generally not seen. For example, in the spontaneous classic murine models of lupus, death usually results from an immune-complex glomerulonephritis with glomerular fibrosis. However, the glomerular morphologic alterations in these mice are characteristically subacute to chronic obliterative in nature with the progression to glomerular fibrosis requiring 25–96 wk depending on the mouse strain (79, 80). The severity of this nephritis is clearly much less than that seen with 11F8.

Along similar lines, adoptive or passive transfer of either anti-DNA hybridoma cells or anti-DNA mAbs has to date only resulted in mesangial immune deposits with a subsequent mild mesangial glomerular hypercellularity (6, 19, 33). For example, Vlahakos et al. report that anti-DNA hybridomas isolated from MRL-*lpr* mice induce intranuclear, linear capillary, and mesangial deposits (19). Some capillary wall subendothelial deposition may be present, but only to a limited extent. In addition, wire-loop lesions are not observed, and the degree of glomerular proliferative changes is not pronounced. To our knowledge, the only other mAb that induces severe glomerulonephritis upon adoptive transfer is 6-19 (61). However, this mAb is an IgG3 rheumatoid factor with cryoglobulin activity so that the relevance of 6-19 to anti-DNA models of glomerular injury is unclear. Thus, adoptive transfer of 11F8 affords a murine model of anti-DNA-induced glomerular injury of the severity observed in many lupus patients. It should be noted that our findings do not preclude the existence either of other nephritogenic anti-DNA like 11F8 or of strongly pathogenic mAbs with other specificities (17).

Pathogenicity: mechanistic studies. A widely held view of pathogenic anti-DNA includes, restricted idiotype, an IgG class, a basic isoelectric point, and cross-reactivity to non-DNA antigens (3, 6, 11–20, 24). Furthermore, it is generally believed that pathogenic anti-DNA possess the capacity to bind dsDNA (6, 11, 24, 27, 33, 81). Based on these criteria, mAb 4B2, which is an IgG2a anti-DNA that binds both ssDNA and dsDNA is a priori the most likely mAb in our panel to be pathogenic. However, this clone is the least pathogenic of the mAbs we tested whereas 11F8, a neutral (pI = 7.5) monospecific anti-ssDNA (35) is the most pathogenic. Of 27 pathogenic anti-DNA mAbs, 11% bind dsDNA, 59% recog-

nize both ssDNA and dsDNA, and 30% are specific for ssDNA (11–16, 23–33). These results do not support a strict relationship between the presence of anti-dsDNA and disease pathogenesis. Instead, the evidence suggests that both anti-ssDNA and anti-dsDNA may participate in disease pathogenesis (82) and may help to explain the occurrence of nephritis in patients that do not possess detectable levels of anti-dsDNA (3, 83, 84).

It was first proposed that localization of pathogenic anti-DNA within glomeruli occurs by passive trapping of circulating anti-DNA–DNA immune complexes (4–6). However, this process has been questioned because of the difficulty in observing anti-DNA–DNA complexes in circulation (9, 10). Alternatively, it has been suggested that pathogenic anti-DNA cross-react with glomerular antigens other than DNA or protein–DNA complexes (6, 12–20). However, both the extent of anti-DNA cross-reactivity and the physiological significance of cross-reactivity measured in vitro is controversial (16, 85, 86). More recently, cogent data have been advanced from several groups demonstrating that anti-DNA form complexes with DNA and/or protein–DNA complexes planted within the GBM (16, 17, 24, 33, 86). Our indirect immunofluorescence experiments showing that a strongly pathogenic mAb like 11F8 apparently binds DNA or DNA-containing molecules trapped in glomeruli more tightly than a weakly pathogenic mAb like 9F11 lends credence to this hypothesis. That DNA is specifically being recognized is supported by the observation that binding to glomeruli is inhibited by DNase I (17, 33, 87).

Why does 11F8 bind "glomerular DNA antigens" whereas 9F11 apparently does not, even though both mAbs apparently possess very similar binding properties in vitro (35), i.e., what is the physical basis for the differential pathogenicity of 11F8 and 9F11? Initial characterization of 11F8 and 9F11 used ssDNA homopolymers, and such test antigens certainly cannot represent the range of all possible ssDNA sequences. If anti-ssDNA possess a considerable degree of sequence selectivity, and the appropriate sequence(s) are planted/adhere to the GBM, sequence-selective DNA binding could lead to localization of one mAb in preference of another. In preliminary support of this hypothesis, we have identified high affinity monovalent consensus sequences for both 11F8 and 9F11 using in vitro selection protocols (Steven, S.Y., and G.D. Glick, unpublished observations; Herrmann et al. have reported similar experiments using SLE sera; 88). Not only are the two consensus sequences different, but 9F11 binds the 11F8 consensus weakly and vice versa. The potential for discriminating between anti-DNA based on selective recognition of a particular DNA antigen may hold significant implications for both the diagnosis and treatment of lupus nephritis. We are currently searching for antagonists that inhibit binding to this antigen.

In summary, we have characterized the V gene usage and structure of eight anti-DNA mAbs as well as the ability of three of these mAbs to induce nephritis in nonautoimmune mice. Combining genetic analysis of these mAbs with previous epitope mapping and equilibrium binding studies (35) has yielded new insight into the relationships between anti-DNA structure and reactivity. Adoptive transfer of 11F8 induces glomerulonephritis in nonautoimmune mice, the severity of which has not previously been observed in murine models of anti-DNA induced glomerulonephritis. This finding provides new information regarding the reactivity and physicochemical properties of pathogenic anti-DNA (6, 24, 89) and suggests that future studies of anti-ssDNA should help to elucidate the nature of anti-DNA–induced renal damage in SLE.

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