Amino Acid Sequence of a Platelet-Binding Human Anti-DNA Autoantibody

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The complete amino acid sequences of the variable regions of the heavy and light chains of a human IgM monoclonal platelet-binding autoantibody have been determined. This antibody, HF2-1/17, produced by a human x human hybridoma prepared from lymphocytes of a patient with systemic lupus erythematosus and thrombocytopenia, is polyreactive with single-stranded DNA, synthetic nucleotides, sulfated carbohydrates, and acidic glycolipids isolated from platelet membranes. The heavy chain is of the V \(_\text{H} \) III subgroup, and the light chain is of the V \(_\text{L} \) I subgroup. The heavy chain is the expression product of the VH26 germline gene. The light chain bears significant homology to other immunoglobulins of known primary structure, including WEA, GAL, HAU, HK101, and DEE. These results suggest that HF2-1/17 may be an autoantibody derived with little or no modification from germline genes. A model of the antibody combining site suggests that arginine 24 and arginine 30 in the light chain (CDR1) interact with a surface defined by phosphate or sulfate groups of the antigen.

Materials and Methods

Preparation of human monoclonal anti-DNA autoantibody HF2-1/17. The preparation of human-human hybridomas from lymphocytes of patients with SLE and the GM4672 parent cell line has been described. Hybridoma cells were grown in vertical 175 cm² flasks (Falcon, Lincoln Park, NJ) and 1 L spinner flasks containing RPMI 1640 (GIBCO, Grand Island, NY), 50 mmol/L HEPES, 20 mmol/L L-glutamine, 10% (vol/vol) fetal calf serum (FCS), gentamicin (20 mg/L), penicillin G (200 U/mL), and streptomycin sulfate (20 mg/L); pH 7.4. Tissue culture supernatant was harvested every 4 to 5 days. A cell density of 2 x 10⁶ cells/mL was maintained. Tissue-culture supernatant was spun at 200 g to remove cell debris; antibody was precipitated with ammonium sulfate (313 g/L). After stirring overnight at 4°C, the precipitate was sedimented by centrifugation at 3,000 g for one hour. The resulting pellet was dissolved in a minimum volume of Tris-buffered saline (TBS; 0.14 mol/L NaCl, 0.5 mol/L Tris HCl, pH 7.4) and dialyzed thrice against 4 L of TBS. IgM was purified by immunoaffinity chromatography using an anti-μ agarose column as described. After the column was washed with TBS, nonspecifically bound proteins were eluted with TBS containing 1 mol/L NaCl. 0.1% Tween 20. After further washing with TBS, the column was eluted with 4 mol/L guanidine HCl (Schwarz/Mann, Cleveland). The eluent was dialyzed against 4 L of TBS twice and evaluated for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12% gels. The antibody was usually about 90% pure. If further purification was required, the IgM was concentrated by ultrafiltration with a PM30 membrane (Amicon, Danvers, MA) to 5 mg/mL and purified on a Biogel A5m column (2.5 x 100 cm) equilibrated in TBS.

Preparation of proteolytic fragments. Fv fragments of the IgM were prepared by the method of Lin and Putnam. The IgM solution was concentrated to 10 mg/mL by ultrafiltration on a PM 30 membrane (Amicon) and then dialyzed against 0.02 mol/L sodium acetate, 0.15 mol/L NaCl, pH 4.0. Pepsin (Sigma Chemical Co, St Louis; 1:25 wt/wt) was then added to the IgM solution and incubated overnight at 4°C. After 24 hours, pepsin (1:50 wt/wt) was again added to the solution and incubated for an additional 24 hours under the same conditions. The digest was neutralized with 10 mol/L NaOH and dialyzed overnight against 4 L of TBS using Spectropor 3 dialysis tubing (exclusion limit mol wt 3,500). The Fv was purified on a Sephadex G-100 superfine column (1 x 100 cm) equilibrated in TBS.
Purified Fv was reduced and alkylated. The reducing buffer solution was made 0.5 mol/L in Tris HCl, 6 mol/L in guanidine HCl, 5 mmol/L in EDTA, pH 8.2. The solution was bubbled under N₂ for two hours, then β-mercaptoethanol was added to a final concentration of 0.12 mol/L. The protein was dissolved in the reducing buffer to yield a concentration of 10 mg/mL and the solution incubated at 23°C for two hours. The protein was alkylated for 45 minutes by the addition of iodoacetamide (Sigma) to a final concentration of 0.14 mol/L. After 45 minutes, sufficient β-mercaptoethanol was added to make the solution 0.18 mol/L, thus quenching alkylating agent that had not reacted with protein. The protein was either dialyzed against 0.025% trifluoroacetic acid or desalted on a Brownlee reverse phase guard column BO3-GU, lyophilized and stored at -80°C.7

Cyanogen bromide digestion of Fv was performed using standard methods.8,9 The dry Fv (100 μg) was dissolved in 70% trifluoroacetic acid containing CNBr and Fv at a 10:1 (wt/wt) ratio at the final protein concentration of 10 mg/mL. The digest was incubated in the dark at 23°C for 18 hours, then a tenfold excess of water over the reaction volume was added and the digest lyophilized. The fragments were purified by high-performance liquid chromatography (HPLC) using a Brownlee reverse phase guard column BO3-GU and a 0.1% TFA-acetonitrile/0.1% TFA gradient.

Trypsin was also used to prepare fragments of the Fv region. Reduced and carboxymethylated Fv (100 to 200 μg) was dissolved in 100 mmol/L Tris HCl at pH 8.0 to yield a final concentration of 5 mg/mL. TPCK-treated trypsin (Worthington, Freehold, NJ) was added at a ratio of 1:100 (wt/wt) of trypsin:Fv. The digest was incubated for 12 to 16 hours, then chromatographed by reverse phase HPLC or stored at -80°C.8

Submaxillary protease digestion was performed by the method of Schenken et al.12 A 1% solution of NH₄HCO₃ (pH 8.0) was added to Fv (100 μg) to yield a final protein concentration of 5 mg/mL. The protease was used at a ratio of 1:50 (wt/wt) enzyme:Fv in two separate additions separated by 12 hours. The digest was incubated at 37°C for a total of 36 hours. The digest was either chromatographed by reverse phase HPLC or stored at -80°C.

Fv fragments were also digested with staphylococcal protease V8.13 Reduced and carboxymethylated Fv (100 μg) was dissolved in 50 mmol/L potassium phosphate, pH 7.8, 2 mmol/L EDTA. Staphylococcal protease (Boehringer Mannheim, Indianapolis) was added in an enzyme to substrate ratio of 1:30 (wt/wt), and the solution was incubated for 24 hours at 34°C. The digest was chromatographed by reverse phase HPLC or stored at -80°C.

**Purification of the proteolytic fragments.** Proteolytic fragments were separated by HPLC. Peptides were detected by monitoring eluate optical density at 214 and 280 nm simultaneously. Enzyme digests of the Fv were chromatographed on a Beckman ODS column (0.46 x 25 cm). A linear gradient was developed at 1%/minute at a flow rate of 0.5 mL/minute. Buffer A was 0.1% aqueous TFA and Buffer B was 0.1% TFA in acetonitrile. Peaks were collected, and those requiring further resolution were diluted twofold in buffer A and rechromatographed under the same conditions except that the gradient was developed at 0.25%/minute from a 0%β of 15% lower than that of the original peak to a %B 10% higher. CNBr fragments were purified as described above except a Brownlee Aqueapore butyl guard column was used.

**Synthesis of the peptide H73-76.** An Applied Biosystems model 430A peptide synthesizer was used to synthesize fragment (73-76) of the heavy chain. The standard Applied Biosystems program was used without modification. The peptide was cleaved from the resin by incubating the complex at 0°C for one hour using hydrogen fluoride containing 10% anisole (vol/vol anisole/HF). The cleaved peptide was twice washed in 400 mL of diethyl ether and twice extracted with 40 mL of 10% acetic acid. The crude peptide was lyophilized and purified by reverse phase HPLC.

**Sequencing of peptides.** Peptides were sequenced using an Applied Biosystems model 470A gas phase protein sequencer online with an Applied Biosystems model 120 PTH analyzer.14 Peptides were loaded onto a glass filter containing 3 mg of polybrene (Sigma). Typically 40 to 500 pmol of peptide were degraded from solutions transferred directly from the HPLC fractions. Alternatively, peptides were concentrated on a Savant Speedvac prior to sequencing.

**Molecular model of HFT2-1/17.** A three-dimensional model of HFT2-1/17 was constructed based upon the known crystal structure of the immunoglobulin McPc603.15 As previously described, the amino acid sequence of 1/17 was aligned with the sequence of McPc603 using EDISEQ.16,17 Amino acids from 1/17 were placed on the McPc603 backbone in identical positions. Residues missing in 1/17 (deletions) were deleted from the model. Additional residues found in 1/17 but not McPc603 (insertions) were inserted into the polypeptide backbone of the model to yield the proper covalent structure. The 1/17 model was modified by the addition of hydrogen atoms and the potential energy was minimized using CHARMM18 on a Star array processor. Under the conditions used, the secondary structure of the backbone of McPc603 was preserved, and minimization was used primarily to eliminate van der Waals overlap. The image was examined and further refined on a Silicon Graphics IRIS 3130 workstation using QUANTA and CHARMM (Polygen, Waltham, MA).

**RESULTS**

The HFT2 1/17 autoantibody was purified from the ammonium sulfate precipitate obtained from tissue culture supernatant by immunofluorimetry using an anti-μ-agarose column and the Edman degradation performed on proteolytic fragments of the isolated Fv region. Typically, 20 to 30 mg of the IgM was recovered from 4 L of supernatant. The protein was usually 90% pure by PAGE analysis in SDS.

**Preparation of the Fv region.** The Fv region was recovered from a cold pepsin digest by gel filtration on a Sephadex G-100 superfine column. The Fv migrated as a doublet of approximately mol wt 14,000 on SDS-PAGE in the presence or absence of β-mercaptoethanol. These results indicate the absence of an interchain disulfide bond connecting the heavy chain and light chain components of the Fv. The recovery of the Fv region was 25% to 30% of the theoretical yield.

**Sequencing strategy.** Because the V₅ and V₇ domains of the Fv could not be readily separated by reverse phase HPLC in good yield, proteolytic fragments for automated Edman degradation were generated from the intact Fv. Reduced and carboxymethylated Fv was digested with submaxillary protease, trypsin, staphylococcal protease V8, and CNBr. The resultant peptides were purified by reverse phase HPLC and then sequenced. In addition, unreduced Fv was digested with CNBr. One of the CNBr fragments was subsequently digested with trypsin. The entire amino acid sequences of both the V₅ and V₇ were determined from the proteolytic fragments of the Fv, except for the light chain where the NH₂-terminus of the purified V₅ was sequenced for 50 residues.

**Results of sequence analyses:** **Heavy chain.** The reduced Fv region was digested with CNBr to yield two dominant
peaks, one consisting of purified fragment (1-34) of the heavy chain and the other consisting of unresolved CNBr fragments derived from the heavy and light chain variable regions. The sequence of CNBr I, fragment (1-34)H, was determined without complication. CNBr digestion was repeated using unreduced Fv region. This mixture was separated by HPLC into four fractions; one peak included CNBr II, fragment (35-83)H. Its sequence was established to cycle 36 (residue 70) by automated Edman degradation. This CNBr fragment was subjected to trypsin digestion and the tryptic peptides resolved by HPLC. Residues 73-83 were established from the analysis of the tryptic digest of CNBr. The tryptic peptides resolved by HPLC. Residues 73-83 were synthesized a peptide corresponding to residues 73-76 of the chain sequence to the VH26 germline gene sequence,9 we attempts to identify it on the HPLC chromatogram. Given separated by HPLC into four fractions; one peak included CNBr II, fragment (35-83)H. Its sequence was established to cycle 36 (residue 70) by automated Edman degradation. This CNBr fragment was subjected to trypsin digestion and the tryptic peptides resolved by HPLC. Residues 73-83 were established from the analysis of the tryptic digest of CNBr II. These fragments included CBII/T1 (68-72)H, CBII/T2 (73-76)H, and CBII/T3 (77-83)H. One of the fragments, from residue 73-76, proved to be especially elusive in attempts to identify it on the HPLC chromatogram. Given the preliminary indication of the relationship of the heavy chain sequence to the VH26 germline gene sequence,15 we synthesized a peptide corresponding to residues 73-76 of the predicted amino acid sequence of VH26. The retention time of this peptide, Asp-Asn-Ser-Lys, was examined and used as a marker to identify a peak in the trypsin digest of the CNBr II fragment with the same retention time as the synthetic peptide. The synthetic fragment eluted on a Beckman ODS column in 100% buffer A after the salt elution. A trypsin fragment CBII/T2, (73-76)H, derived from the CNBr fragment had an identical retention time as the synthetic peptide; the sequence of this trypsic fragment revealed residues 73-76.

CNBr digestion of the reduced Fv region included a complex peak containing fragments (35-83)H and (84-x)H (x connotes the undetermined COOH-terminal residue) of the heavy chain. Fragment (84-x)H could not be completely resolved from fragment (35-83)H. Therefore this mixture was cosequenced. The sequence of (84-x)H was deduced by subtracting out the known fragment (35-83) sequence. The sequence of this fragment was established through cycle 21 (residue 105).

Five fragments produced from a submaxillary protease (SMP) digest of the reduced and alkylated Fv region were used to complete the sequence of the heavy chain. These included fragments SMP 19 (80-87)H, SMP 34 (88-96)H, SMP 55 (110-112)H, SMP 39 (110-122)H, and SMP 44 (113-125)H (Fig 2). Fragment SMP 44 (113-125)H was sequenced up to the constant region. The sequence of other proteolytic fragments, including trypptic fragments T19 (1-19), T68 (44-65), T8 (20-32), T35 (88-98), and T37 (88-96), submaxillary protease fragment SMP 32 (1-19), and staphylococcal protease fragments SP 39 (2-6) and SP 68 (7-19) provided further validation of the sequence depicted in Fig 2. With the exception of residues 72-73 and 76-77, overlapping peptides allowed the unambiguous alignment of all other fragments to establish the heavy chain sequence. Comparison of the sequence with the framework sequence of other V_H sequences,20 the predicted protein sequence of the VH26 germline gene,14 and the nucleotide sequence of 1/17 heavy chain21 confirmed this alignment.

Results of sequence analyses: Light chain. The NH2-terminal 50 amino acids of the light chain were determined by automated degradation of the isolated light chain variable region derived from the Fv preparation. The remaining sequence of the light chain was determined by digesting the reduced Fv with staphylococcal protease V8, submaxillary protease, or trypsin (Fig 3). SMP was contaminated with a chymotryptic-like activity; many of the SMP fragments were cleaved after aromatic and hydrophobic amino acids. Nine SMP peptides were used to identify sequences in the light chain, including SMP 39 (25-30), SMP 36 (25-36), SMP 33 (31-36), SMP 30 (46-50), SMP 22 (62-72), SMP 37 (72-83), SMP 28 (89-96), SMP 26 (97-106), and SMP 32 (97-108). Of the nine fragments, two (SMP 36 and SMP 30) contained arginine residues that were not cleaved by SMP.
1/17 Heavy Chain

Fig 2. Variable region amino acid sequence of the heavy chain of HF2 1/17. Complementarity determining regions (CDR) are indicated. The overlapping sequences employed to determine and to align the heavy chain sequence are labeled. These data represent the experimentally derived sequences obtained from each fragment but not necessarily the entire sequence of the fragment. The numbering system of Kabat is used.

1/17 Light Chain

Fig 3. Variable region amino acid sequence of the light chain of HF2 1/17. CDR are indicated. The overlapping sequences employed to determine and to align the light chain sequence are labeled. These data represent the experimentally derived sequences obtained from each fragment but not necessarily the entire sequence of the fragment. The constant region begins at residue 108. The numbering system of Kabat is used.
Trypsin digestion generated fragments from the Fv region. Five fragments were identified in the light chain, and four fragments [T50 (31-42), T49 (47-61), T72 (62-90), T44 (88-103), and T54 (109-116)] contributed to the sequence of the variable region of the light chain. As seen in Fig 3, the order of the SMP and tryptic peptides is defined by overlap-staphylococcal protease V8 fragments SP 27 (1-17), SP 38 (71-81), SP 46 (82-92), SP 60 (82-105), and SP 30 (106-114).

Structure of HF2-1/17. To gain insight into the general location of basic amino acids in the antibody-combining site that might participate in recognition of various negatively charged antigens, a computer model of 1/17 was prepared based upon the known primary structure of the variable regions of the heavy and light chain. This model is based upon the three-dimensional structure of the polypeptide backbone of McPc603.26 The variable regions of human IgM HF2-1/17 demonstrate 67% sequence homology with the murine McPc603. As aligned in Fig 4, a model of HF2-1/17 based upon McPc603 required one deletion in both the heavy chain (two residues) and light chain (six residues) of the McPc603 backbone. The model also required two insertions, one of three residues and one of two residues, in the heavy chain. The model of HF2-1/17 is identical to that of McPc603 except for the substitution of side chains of HF2-1/17 for those of McPc603 and the ambiguity of the precise position of residues in and adjacent to the insertions and residues adjacent to deletions. Energy minimization was employed on the side chains of these residues to eliminate van der Waals overlap. Thus with the exception of perturbations of the polypeptide structures adjacent to these insertions and deletions, the backbone structure of 1/17 is essentially identical to that of McPc603. Although this model lacks the precision of an x-ray-derived structure, it does provide a first approximation of the general surface location of amino acids in the complementarity determining regions.

The antibody combining site of 1/17 was examined to identify surfaces that potentially interact with the phosphate groups on phospholipids and single-stranded DNA. On the basis of the crystal structure of gene 5 DNA binding protein complexed to ssDNA,22 it has been shown that arginine and lysine side chains may interact electrostatically with the phosphate of ssDNA, while the nucleic acid bases may stack with aromatic residues or interact through hydrogen bonds with tyrosine residues. Figure 6 demonstrates that only two positively charged residues in complementarity determining regions, arginine 24 and arginine 30 on the light chain, are located on the surface of the antibody combining site of 1/17. These residues are likely to define the surface to which ssDNA and platelet glycolipids interact through the phosphate or sulfate groups on the antigen.

DISCUSSION

We have established the complete primary structure of the variable regions of the light and heavy chains of the human monoclonal platelet-binding anti-DNA autoantibody HF2-1/17 and have verified the heavy chain amino acid sequence predicted from the nucleotide sequence.21 This autoantibody is of special interest because it is a prototype of the 16/6 idiootype family of immunoglobulins that occurs in the serum of patients with SLE and that may be responsible for some of the clinical and pathologic manifestations of the disease,21 including thrombocytopenia. Furthermore, this antibody, like others of its type, has binding reactions with several different clinically relevant antigens, including ssDNA,3 synthetic polynucleotides,7 and certain phospholipids, glycolipids, and sulfated carbohydrates.5,6

We have previously demonstrated the structural homology of the NH2-terminal sequences of the heavy and light chains of some of the human monoclonal autoantibodies that express the 16/6 idiootype.7 These studies indicated that the heavy chain includes a V,III subgroup framework7 and that the amino terminal 40 amino acids are identical with the sequence predicted from the VH26 gene.16 The framework region of the light chain is characteristic of the V,i subgroup; the first 40 amino acids are identical with the IgM WEA, a Waldenström's macroglobulin that interacts with a Klebsiella polysaccharide containing 3,4 pyruvylated galactose.6,24 With our current data we are now able to assess the relationship between the variable regions of 1/17 with the complete VH26 sequence and with the complete WEA light chain sequence.

The sequence of the light chain variable region is in agreement with the partial sequence of HF2-1/17 previously presented.7 With the exception of residue 28, the sequence of the heavy chain variable region is also in agreement with the partial sequence of this chain in HF2-1/17.7 Sequence analyses of CNBr I, (1 to 34)H, and T8, (20 to 32)H, revealed a threonine at cycle 28 and cycle 9, respectively, indicating a threonine and not a lysine at residue 28 in the heavy chain. Furthermore, nucleotide sequence analysis of the RNA coding for 1/17 predicts the heavy chain sequence presented in Fig 3.21

Based upon partial sequences of the variable regions, Atkinson et al7 had previously shown the marked structural homology among the heavy and light chains of the 16/6-idiootype-positive IgM autoantibodies. This observation has now been extended by Dersimonian et al,25 who have shown that the nucleic acid sequences of the heavy chains of 18/2 and 1/17 are identical to each other and to the VH26 germline gene,19 and to 30P1,25 a fetal B-cell clone. The primary structure of the heavy chain of 1/17, obtained by direct protein sequencing in this work, confirms the predicted amino acid sequence derived from the nucleotide sequence of the 1/17 heavy chain.21 The identity of the HF2-1/17 heavy chain to the germline-encoded VH26 and 30P1 heavy chain is comparable with the finding of Radoux et al26 and Chen et al27 that the light chains of human rheumatoid factors are encoded by Vc genes that are identical to or closely related to germline VL genes. The heavy chain of the murine (MRL/lpr/lpr) lupus anti-DNA autoantibody H130 is also encoded by an unmutated germline gene.28 The Jc region of the heavy chain of 1/17, which differs by two amino acids from the primary structure predicted by the Jc5 germline gene, is encoded by a mutated form of the Jc5 germline gene or related germline gene.29 These results suggest that the germline immunoglobulin repertoire includes the potential for expression of autoantibodies. Antibodies that are idio-
SEQUENCE OF AN ANTI-PLATELET LUPUS AUTOANTIBODY

**Light Chain**

1/17  DIQMTQSPSSASVGRVTITCRASQGRND  LGWQQKPGKAKRILYAASSLQGVPFSRSGSGETLTISSLQEDFATYYCLQNSYPLTFGGTK

McPc603  -V-----V-A---MS-KS--SLL-SGNYKNF-A-------QP-L-G-TRE----D-T-----D------V--L-V---QNH------A-

**Heavy Chain**

1/17  EVQLVESGGGLVQPGGLRLSCAASGFTSSYAMSRVQAPGKLESWYSASGSGG  STYYADSVKGRFTISDNGKNTLYQLMNSLRAEDTAVYYCAGQYLVYGSYHFDWGGTLTVSS

McPc603  -K-V----------T-------DFY-E-----P-----IA-SRNK-NKYT-E-SA------IV-T-OSI------A----------R  TMY-V-A--T-------

**Fig 4.** Comparison of the variable regions of the light and heavy chains of HF2 1/17 and McPc603. The sequences have been aligned for maximum homology.

**Fig 5.** Comparison of the variable regions of the light chain of HF2 1/17 and other related light chains. HF2 1/17 and five light chain variable regions are aligned for maximum homology. Significant similarity is observed among the framework regions of 1/17, WEA, GAL, Hau, HK101, and Dee, while the CDR1 of 1/17 is homologous to WEA and GAL, and the CDR2 of 1/17 is homologous to WEA, GAL, Hau, HK101, and Dee.
typically related to 16/6 and to H130 have been identified in the renal lesions of human and murine lupus. The latter study, which involved MRL/lpr/lpr mice, showed that H130-related antibodies were ten times more concentrated in nephritic kidneys than in serum. It is therefore plausible that these families of lesion-associated autoantibodies arise from unmutated germline genes in both humans and murine lupus.

The sequence of the variable region of the light chain of IgM 1/17 is not identical to any immunoglobulin of known primary structure. As a k light chain, this sequence bears some homology with five human kappa light chains whose sequence has been previously determined (Fig 5). WEA, a Waldenström’s macroglobulin that binds to pyruvylated galactose and ssDNA, expresses the 16/6 idiotype. IgM 1/17, also a Waldenström’s macroglobulin, shares a common CDR1 with both WEA and GAL, with the exception that 1/17 contains a glycine at residue 34, while WEA and GAL contain a threonine. The HK101 sequence, derived from a germline gene exhibits a CDR2 that is identical to 1/17. Hau, Dee, and GAL have CDR2 regions that are homologous to 1/17 but have a single mismatch at residue 55, residue 55, and residue 53, respectively. WEA differs at residue 50 and residue 52. The J region of the light chain is encoded without substitution by the J,4 germline gene.

In the absence of a crystal structure of the HF2-1/17, the model of the combining site of 1/17 permits a first approximation of amino acids that may define the polyreactivity of this antibody with numerous antigens, including platelet glycolipid and single-stranded DNA. Arginine 24 (CDR1L) and arginine 30 (CDR1L) are the only residues of positive charge in the complementarity determining regions of the heavy and light chains of 1/17. These residues, exposed on the surface of the antibody combining site (Fig 6), likely interact with the negatively charged phosphate groups on polynucleotides or sulfate groups on some platelet glycolipids. Arginine is the most common amino acid observed at residue 24 of the human k light chain. Serine is the most common amino acid observed at residue 30 of these light chains, but arginine is also observed at high frequency. However, the only k light chains containing arginine residues at both residue 24 and residue 30 are 1/17, 16/6, 18/2, 21/28, WEA, and GAL. All these light chains are components of anti-DNA binding antibodies. These results suggest a correlation between the DNA-binding properties of these antibodies and the presence of arginine at residues 24 and 30. The chemical similarity between ssDNA and the platelet autoantigen is recognized by HF2-1/17. The chemical structure of the platelet autoantigen is currently under investigation, but we anticipate that the structural elements in this glycolipid will resemble those of polynucleotides.

ACKNOWLEDGMENT

We wish to thank Dr Kathleen Barrett for many helpful discussions and for bringing to our attention, on the basis of our prior work, the relationship of the heavy chain primary structure and the DNA sequence of the VH26 germline gene. We are also grateful to Richard Feldmann for his assistance in the early phases of the model building.

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Amino acid sequence of a platelet-binding human anti-DNA monoclonal autoantibody

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