# Cardiolipin Binding a Light Chain from Lupus-Prone Mice[†,‡]

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ABSTRACT: Autoantibodies in systemic lupus erythematosus react with multiple epitopes on highly conserved molecules such as nucleic acids, cytoskeletal proteins, phospholipids, and phospholipid-binding proteins. Analysis of the heavy- and light-chain variable sequences (VH and VL) has shown that a restricted set of V genes gives rise to these autoantibodies. Several monoclonal antibodies were developed from a strain of mouse prone to lupus (F1 male NZW × BXSB). Two of these antibodies, A1.72 and A1.84, reacted directly with cardiolipin and their VH and VL sequences were analyzed. Surprisingly, these two antibodies had identical light-chain variable sequences despite having substantially different heavy-chain variable sequences. This VL sequence, VL 72/84 was 97% identical with the germ-line sequences with only four single nucleotide substitutions. When this VL sequence was shuffled with the VH sequence of other monoclonal antibodies and expressed as single chain variable fragment (scFv) in *Escherichia coli*, it imparted cardiolipin-binding activity to the hybrids. Furthermore, the VL 72/84 sequence, when expressed alone without any VH sequence, also bound to cardiolipin. The antibodies and their recombinant fragments were immunoaffinity-purified on cardiolipin liposomes. The dissociation constant of the light chain for cardiolipin was similar to the intact molecule (21 ± 0.01 vs 20 ± 0.03 nM). These studies demonstrate that the VL sequence alone, in the absence of any other immunoglobulin domains, can mediate cardiolipin binding, raising the possibility that antigen specificity of certain antibodies may exclusively reside in their light-chain sequences.

Patients with systemic lupus erythematosus and other autoimmune disorders develop antibodies directed against constituents of normal cells, including macromolecules such as DNA, cytoskeletal proteins, phospholipids, and phospholipid-binding proteins (*1*−*4*). Although the presence and titers of some of these autoantibodies correlate with the activity of the disease, their origin and pathogenic role are not clearly understood. Analysis of a large panel of monoclonal autoantibodies in murine and human lupus has revealed that most of them originate from a restricted set of V genes and in many cases are coded by germ-line sequences (*5*−*8*).

Antibodies to cardiolipin are a major subset of these autoantibodies. Recent studies have shown that the epitopes for the majority of these antibodies are present on the complex of cardiolipin and proteins that bind to cardiolipin such as β2-glycoprotein I (*9*−*11*). In contrast, some antibodies interact with cardiolipin in the absence of any other proteins. In addition to lupus, anticardiolipin antibodies are frequently found in conditions associated with infections such as syphilis, Q fever, and infectious mononucleosis (*12*, *13*).

An animal model of systemic lupus erythematosus is provided by the F1 male hybrids of the cross between NZW and BXSB mice (F1 male NZW × BXSB), which develop a lupus-like disease with many autoantibodies similar to those found in human lupus including anticardiolipin antibodies (*14*, *15*). We have developed and sequenced several monoclonal anticardiolipin antibodies from these mice and provide evidence that cardiolipin-binding activity can be mediated solely by a light-chain sequence.

## EXPERIMENTAL PROCEDURES

Chromatographically pure bovine heart cardiolipin (>99% by HPLC) was obtained from Avanti Polar Lipids, Alabaster, AL. Cholesterol, dicetyphosphate and other standard laboratory reagents were obtained from Sigma Chemical Co., St. Louis, MO.

*Generation of Monoclonal Antibodies from Lupus-Prone NZW × BXSB, F1 Hybrid Mice.* The F1 males of the NZW × BXSB cross were obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN. The mice were sacrificed at 3−4 months, when their sera contained anticardiolipin antibodies. The splenic lymphocytes (∼1 × $10^8$) were fused with the BALB/c nonsecreting myeloma cell line NS-1, as described previously (*16*). The hybrid cells were plated in HAT

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[‡] These nucleotide sequences of the antibodies have been submitted to the GenBank and is given accession numbers AF019942−6.

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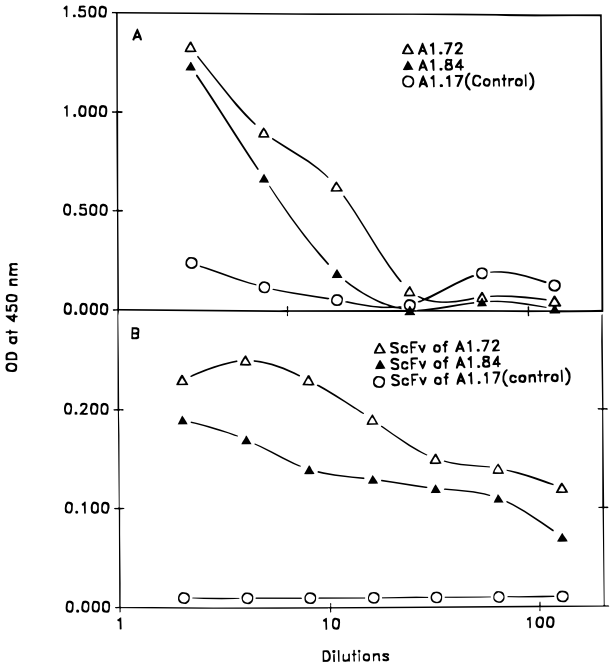



FIGURE 1: Reactivity of the monoclonal antibodies and their recombinant scFvs with cardiolipin: splenic lymphocytes from F1 male NZW × BXSB were fused with the BALB/c nonsecreting myeloma cell line NS-1. Hybrid cells were plated in HAT medium and antibody secreting clones were identified by an ELISA and recloned. The mRNA from each hybridoma cell line was reverse transcribed and the cDNA for the VL and VH sequences was amplified by PCR and cloned into the expression vector pCANTAB E. Competent *E. coli* (strain HB2151) was transformed with pCANTAB E plasmid DNA containing the scFv constructs and selected on plates containing 100 mg/mL ampicillin. Tissue culture fluid from the hybridoma cell line (panel A) and the periplasmic extracts of the corresponding transformed *E. coli* synthesizing scFvs (panel B) were tested for anticardiolipin activity by ELISA. △, A1.72; ▲, A1.84, and ○, control anti-$\beta$2-glycoprotein I antibody.

medium, and the supernatants from hybrid wells were tested for anticardiolipin binding activity by ELISA (see below). Positive hybrids were cloned three times by limiting dilution.

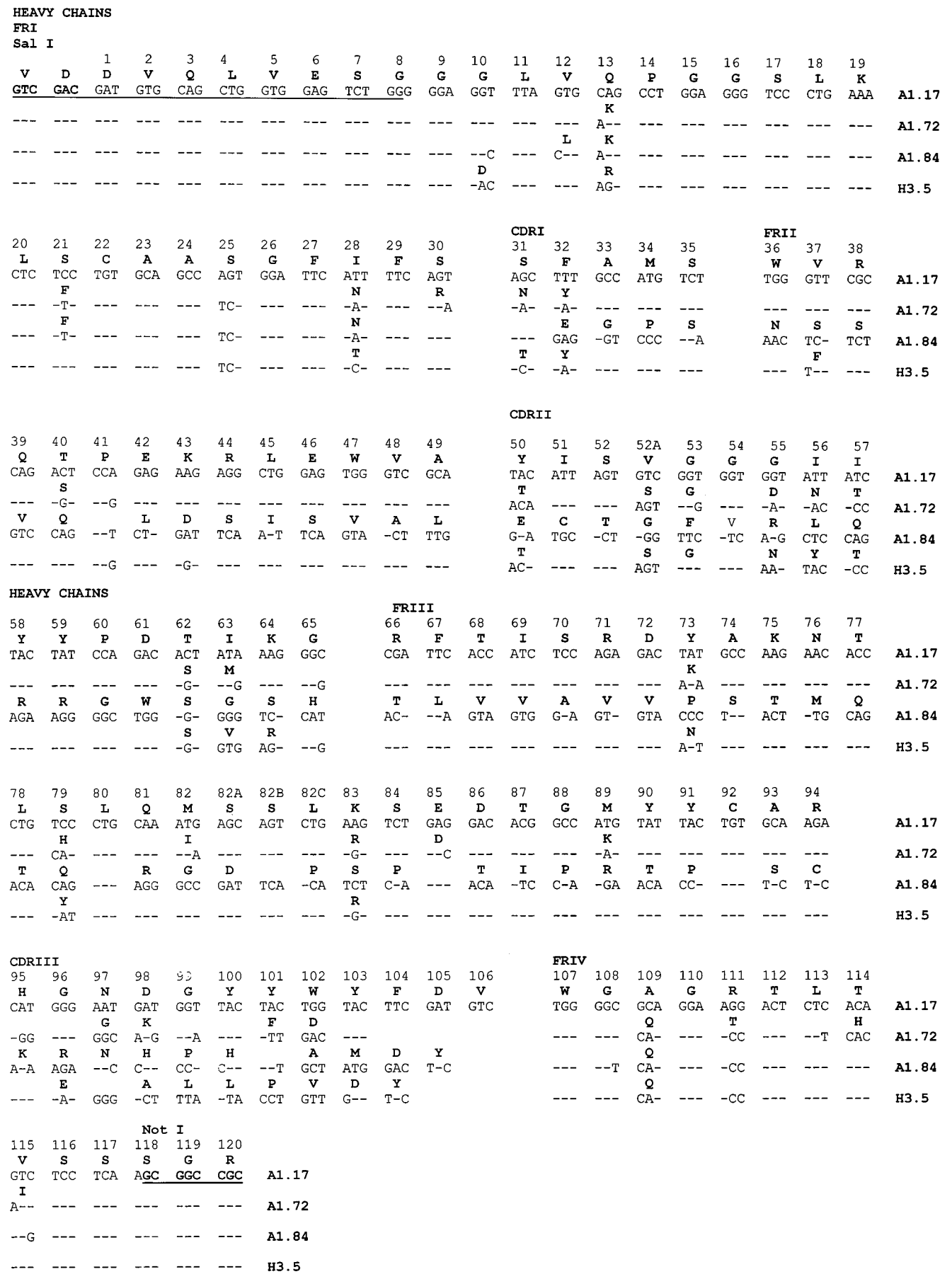
*mRNA Isolation and Single-Chain Fv (scFv) Construction.* The mRNA from each of the individual hybridoma cell lines ($5 \times 10^7$ cells) was isolated using the Fast Track mRNA isolation kit (Invitrogen, San Diego, CA). Nucleotide primers were synthesized based on the Kabat nucleotide database to amplify the cDNA for the VH[1] and VL sequences (*17*). VL-5′ primer contained an *Sfi*I site and 23 nucleotides encoding the N-terminal amino acids of the light chain at its 5′ end. VL-3′ primer contained a *Sal*I site and 36 nucleotides coding for a linker peptide; in addition, its 3′ end was complementary to the joining (J) region of a mouse $\kappa$ light chain. VH-5′ primer contained a *Sal*I site and 20 nucleotides encoding the N-terminal amino acids of the heavy chain. VH-3′ primer contained a *Not*I site and its 3′ end was complementary to the J region of the heavy chain. The mRNA used for first strand cDNA synthesis was heated to 70 °C for 10 min and cooled on ice for 2−5 min before starting the reverse transcription reaction at 37 °C for 1 h. The 20 mL reaction mixture contained approximately ∼1 $\mu$g of mRNA, 1 mM each dNTP, 1 unit/mL of RNasin, 2.5 $\mu$M random primers of oligo dT or 1 $\mu$M VL-3′ or VH-3′ primers, and 50 units of MuLV reverse transcriptase (Perkin-Elmer Corp., Norwalk, CT) in a pH 8.3 buffer, containing 50 mM KCl, 10 mM Tris-HCl, and 5 mM $MgC1_2$. Amplification of VL and VH cDNAs was performed in a 100 mL volume containing 2.5 units of Taq polymerase (Perkin-Elmer Corp., Norwalk, CT), 20 mL of the first strand synthesis reaction mixture, 2 $\mu$M of the primers (either VL-5′ and VL-3′ or VH-5′ and VH-3′ where random primers and oligo dT were used and VL-5′ and VH-5′ in the case where downstream primers were used), 50 mM KCl, 10 mM Tris HCl, and 2 mM $MgCl_2$ (pH 8.3) in a Gene Amp PCR system 9600. After 2 min of a denaturation step at 94 °C, 40 cycles of PCR were carried out under the following conditions: denaturation at 94 °C for 1 min, annealing at 55 °C for 90 s, and polymerization at 72 °C for 2 min, with a 10 s extension in each cycle. Following amplification, a single band of ∼350 bp was seen for both the heavy and light chains.

*Bacterial Expression of Soluble scFv.* The amplified VL sequences were digested with *Sfi*I and *Sal*I, and the amplified VH sequences were digested with *Sal*I and *Not*I and subcloned into the expression vector pCANTAB E (Pharmacia Biotech, Piscataway, NJ). Shuffling of the heavy- and light-chain constructs between the monoclonal antibody V genes was also carried out, and the new constructs were subcloned into pCANTAB E. The expression vector pCANTAB E carries a nucleotide sequence in front of the amber stop codon, coding for a 13 amino acid peptide, GAPVPYPDPLEPR, called E-tag, at its carboxy-terminus. To generate soluble recombinant scFv antibodies, competent *Escherichia coli* strain HB2151 was transformed with pCANTAB E plasmid DNA containing the scFv gene constructs. HB2151 is a nonsuppressor strain where the amber stop codon will be recognized. Transformed HB2151 were plated onto agar containing 100 mg/mL of ampicillin and 2% glucose. Individual colonies were picked and grown overnight in 5 mL of 2XYT containing 100 mg/mL ampicillin and 2% glucose. An overnight culture of transformed HB2151 (5 mL) was transferred to 50 mL of 2 × YT containing 100 mg/mL ampicillin and 2% glucose and grown for another hour at 37 °C. After centrifugation the bacterial pellet was resuspended in 50 mL of 2 × YT medium containing ampicillin, induced with 1mM IPTG and grown at 37 °C on a shaker. After 24 h, bacteria were removed by centrifugation at 1500*g* for 15 min at room temperature. The supernatants contained the extruded extracellular soluble antibody. The pellets were processed further for periplasmic and whole cell extracts. Periplasmic extracts were obtained by resuspending the pellet for 30 min in 0.5 mL of PBS containing 1 mM EDTA on ice followed by centrifugation. The whole cell extract was obtained by resuspending the pellet in boiling PBS for 5 min, followed by centrifugation.

*SDS−PAGE and Immunoblotting.* SDS−PAGE was performed according to the method of Laemmli (*18*). Following electrophoresis the proteins were either stained with Coomassie Blue or transferred to nitrocellulose as described previously (*19*). The nitrocellulose was blocked by incubation (1 h) with 5% nonfat milk in TBS, (Tris-buffered saline, 0.15 M NaCl, 20 mM Tris, pH 7.5) containing 0.1% Tween-20. The blots were washed and incubated (1 h, 22 °C) with horseradish peroxidase-labeled goat anti-mouse $\kappa$ antibody (1:5000, for scFvs and 1:7000

[1] Abbreviations: ScFv, single chain variable fragment; VH, variable heavy chain; VL, variable light chain; TBS, tris-buffered saline.

A


HEAVY CHAINS
FRI
Sal I
V D 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
GTC GAC GAT GTG CAG CTG GTG GAG TCT GGG GGA GGT TTA GTG CAG CCT GGA GGG TCC CTG AAA A1.17
K
--- --- --- --- --- --- --- --- --- --- --- --- --- A-- --- --- --- --- --- --- A1.72
L K
--- --- --- --- --- --- --- --- --- --- --- --C --- C-- A-- --- --- --- --- --- --- A1.84
D R
--- --- --- --- --- --- --- --- --- --- --- -AC --- --- AG- --- --- --- --- --- --- H3.5
CDRI
FRII
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38
L S C A A S G F I F S S F A M S W V R
CTC TCC TGT GCA GCC AGT GGA TTC ATT TTC AGT AGC TTT GCC ATG TCT TGG GTT CGC A1.17
F N R N Y
--- -T- --- --- --- TC- --- --- -A- --- --A -A- -A- --- --- --- --- --- --- A1.72
F N E G P S N S S
--- -T- --- --- --- TC- --- --- -A- --- --- --- GAG -GT CCC --A AAC TC- TCT A1.84
T T Y F
--- --- --- --- --- TC- --- --- -C- --- --- -C- -A- --- --- --- --- T-- --- H3.5
CDRII
39 40 41 42 43 44 45 46 47 48 49 50 51 52 52A 53 54 55 56 57
Q T P E K R L E W V A Y I S V G G G I I
CAG ACT CCA GAG AAG AGG CTG GAG TGG GTC GCA TAC ATT AGT GTC GGT GGT GGT ATT ATC A1.17
S T S G D N T
--- -G- --G --- --- --- --- --- --- --- --- ACA --- --- AGT --G --- -A- -AC -CC A1.72
V Q L D S I S V A L E C T G F V R L Q
GTC CAG --T CT- GAT TCA A-T TCA GTA -CT TTG G-A TGC -CT -GG TTC -TC A-G CTC CAG A1.84
T S G N Y T
--- --- --G --- -G- --- --- --- --- --- --- AC- --- --- AGT --- --- AA- TAC -CC H3.5
HEAVY CHAINS
FRIII
58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77
Y Y P D T I K G R F T I S R D Y A K N T
TAC TAT CCA GAC ACT ATA AAG GGC CGA TTC ACC ATC TCC AGA GAC TAT GCC AAG AAC ACC A1.17
S M K
--- --- --- --- -G- --G --- --G --- --- --- --- --- --- --- A-A --- --- --- --- A1.72
R R G W S G S H T L V V A V V P S T M Q
AGA AGG GGC TGG -G- GGG TC- CAT AC- --A GTA GTG G-A GT- GTA CCC T-- ACT -TG CAG A1.84
S V R N
--- --- --- --- -G- GTG AG- --G --- --- --- --- --- --- --- A-T --- --- --- --- H3.5
78 79 80 81 82 82A 82B 82C 83 84 85 86 87 88 89 90 91 92 93 94
L S L Q M S S L K S E D T G M Y Y C A R
CTG TCC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACG GCC ATG TAT TAC TGT GCA AGA A1.17
H I R D K
--- CA- --- --- --A --- --- --- -G- --- --C --- --- --- -A- --- --- --- --- --- A1.72
T Q R G D P S P T I P R T P S C
ACA CAG --- AGG GCC GAT TCA -CA TCT C-A --- ACA -TC C-A -GA ACA CC- --- T-C T-C A1.84
Y R
--- -AT --- --- --- --- --- --- -G- --- --- --- --- --- --- --- --- --- --- --- --- H3.5
CDRIII
FRIV
95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114
H G N D G Y Y W Y F D V W G A G R T L T
CAT GGG AAT GAT GGT TAC TAC TGG TAC TTC GAT GTC TGG GGC GCA GGA AGG ACT CTC ACA A1.17
G K F D Q T H
-GG --- GGC A-G --A --- -TT GAC --- --- --- --- --- --- CA- --- -CC --- --T CAC A1.72
K R N H P H A M D Y Q
A-A AGA --C C-- CC- C-- --T GCT ATG GAC T-C --- --T CA- --- -CC --- --- --- A1.84
E A L L P V D Y Q
--- -A- GGG -CT TTA -TA CCT GTT G-- T-C --- --- CA- --- -CC --- --- --- H3.5
Not I
115 116 117 118 119 120
V S S S G R
GTC TCC TCA AGC GGC CGC A1.17
I
A-- --- --- --- --- --- A1.72
--G --- --- --- --- --- A1.84
--- --- --- --- --- --- H3.5

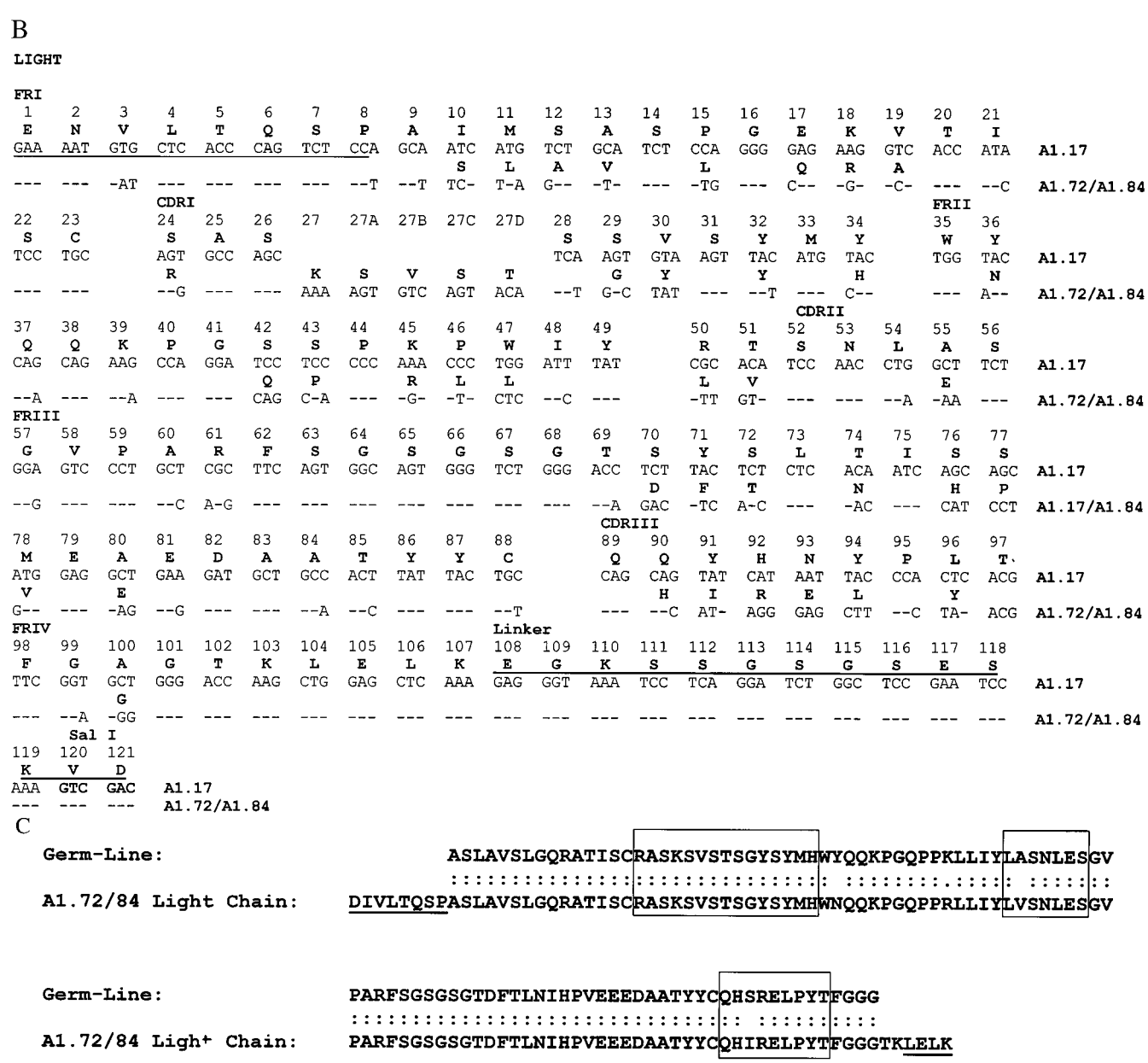



FIGURE 2: V gene analysis of the antibody sequences: (A) nucleotide and the deduced amino acid sequences of heavy chains (from top) VH of A1.17, A1.72, and A1.84 and H3.5; restriction sites and primers are underlined; complementarity determining regions (CDRs) and framework regions are marked in boldface; (B) nucleotide sequences of light chains VL of A1.17 (top); VL 72/84 (bottom); (C) comparison of amino acid sequence of germ-line and deduced light chain sequence of VL 72/84. The CDRs are boxed. These sequences have been submitted to the GenBank and given accession numbers AF019942−6.

for the light chains) for 1 h at room temperature. The blots were washed and soaked for 1 min with a 1:1 mixture of detection reagent 1 and reagent 2 of the ECL Western blotting detection kit (Amersham, Arlington Heights, IL). The excess reagent was drained, and the blots were exposed to Kodak XAR film and developed. The recombinant antibody fragments were also detected using anti Etag antibody as per the manufacturer's instruction (Pharmacia Biotec, Piscataway, NJ).

*ELISA for Cardiolipin.* Microtiter plates (Falcon 3714) were coated with 1 mg of $\beta$2-glycoprotein I or 2.5 mg of cardiolipin as described before (*20*). Plates were blocked with 3% BSA for 1 h after which they were washed with TBS. The supernatants were diluted serially with 1% BSA in TBS and allowed to incubate at room temperature for 1 h. Plates were washed 4 times with TBS and then incubated with horseradish peroxidase-labeled goat anti-mouse IgGk (1:1000 dilution) for 1 h at room temperature. After the plates were washed and peroxidase substrate, orthophenyldiamine (1 mM), and $H_2O_2$ were added, the absorbance was measured at 450 nm using a microplate reader (Molecular Devices Corp. Menlo Park, CA). ELISA was also carried out using a mouse anti-E tag antibody and peroxidase-labeled goat anti-mouse IgG as primary and secondary antibodies for the detection of expressed recombinant antibody.

*Immunoaffinity Isolation of the Antibodies and the Recombinant Fragments.* Multilamellar liposomes were prepared according to the method of Kinsky (*21*). Cardiolipin, cholesterol and dicetyl phosphate were dissolved in chloroform in a molar ratio of 10:15:1 and dried in a stream of

nitrogen in a conical flask. Five milliliters of TBS was added to the flask and agitated vigorously in a vortex mixer for 1 min. The liposomes were washed by centrifugation at 3000*g* for 10 min, and then incubated with the bacterial extract or tissue culture supernatant for 15 min at 37 °C. The mixture was centrifuged at 10000*g* for 10 min, and the pellet was washed three times in TBS and the bound proteins were eluted in 1 M NaI as described before (*22*) and dialyzed in TBS. From 50 mL of bacterial extract, 60 $\mu$g of scFv and 50 $\mu$g of light chains were isolated and from 50 mL of hybridoma tissue culture supernatant 200 $\mu$g of intact IgM was isolated.

*Determination of Dissociation Constants*. The dissociation constants of the isolated antibodies and the recombinant fragments for cardiolipin was determined by Scatchard analysis of an ELISA, as described previously (*23*, *24*). Purified IgM ($4 \times 10^{-10}$ M), scFvs ($5 \times 10^{-10}$ M), and the light-chain fragment ($1.2 \times 10^{-9}$ M) were incubated with increasing amounts of cardiolipin ($8 \times 10^{-9}$ M to $2 \times 10^{-7}$ M) in phosphate buffer for 15 h at 25 °C. The free antibody concentration was determined at equilibrium by transferring an aliquot of this incubation mixture (150 $\mu$L) to an ELISA plate coated with 2.5 $\mu$g of cardiolipin. The plates were washed, and the bound antibody or its fragments were quantified by peroxidase-labeled goat anti-mouse $\kappa$ antibody using orthophenyldiamine as described in the ELISA for cardiolipin antibodies. The free antibody concentration at equilibrium (i), is related to the absorbance (*A*) measured in the ELISA by $i/i_o = A/A_o$, where $A_o$ is the measured absorbance in the absence of antigen and $i_o$ is the total antibody concentration, deduced from a prior calibration in an ELISA. The fraction of the bound antibody ($v$) corresponds to $A_o - A/A_o$ and the concentration of the free antigen ($a$) at equilibrium corresponds to $a_o - i_o'[A_o - A/A_o]$, where $a_o$ is the total concentration of antigen, while $i_o'[A_o - A/A_o]$ is the bound antibody. The dissociation constants for the antibodies and scFvs and the light chain were determined on three separate occasions.

The liver DNA from the mouse was isolated as described before (*25*). The genomic DNA was amplified using two sets of PCR primers so that overlapping segments of the DNA were isolated. The primers used were G1F, 5′-TCCTGCAGGGCCAGCAAAAGTGTCAGTACA-3′ (forward primer) and G1R, 5′-CCCCCCTCCGAACGTGTAGGGAAGCTCCCT-3′ (reverse primer) and G2F, 5′-GTACTCACCCAGTCTCCTGCTTCCTTAG-3′ (forward primer) and G2R (reverse primer). DNA sequencing was carried out on an automated DNA sequencer, ABI Prism (Applied biosystem, Foster City, CA).

## RESULTS

*Specificity of the Monoclonal Antibodies*. Several stable hybridoma cell lines were established from F1 male NZW × BXSB mice. Two of these antibodies, A1.72 and A1.84, reacted with cardiolipin in ELISA in the absence of any serum proteins (Figure 1, panel A). These hybridoma-derived antibodies were also expressed as single-chain variable fragments (scFv) in *E. coli*. These recombinant scFvs had the same specificity as the parent hybridoma cell line, showing that their interaction with cardiolipin was through the V region (Figure 1, panel B).

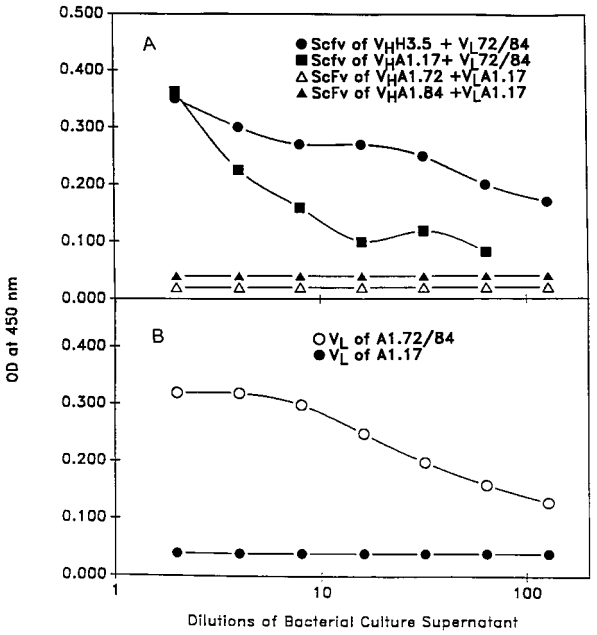



FIGURE 3: (A) Effect of shuffling the light chain on the reactivity toward cardiolipin. The common variable light chain, VL 72/84, was shuffled with the VH sequence of H3.5 (anti-vitronectin) and the VH sequence of A1.17 (anti-$\beta$2-glycoprotein I) separately, and the resulting scFvs were tested in ELISA assays. ●, scFv of VH H3.5 + VL 72/84; ■, scFv of VH A1.17 + VL 72/84; △, scFv of VH A1.72 + VL A1.17; ▲, scFv of VH A1.84 + VL A1.17. (B) Cardiolipin binding activity of the recombinant light chain proteins: the VL sequences of A1.72/1.84 and A 1.17 were expressed in *E. coli* and tested for cardiolipin activity by ELISA. ○, VL of A1.72/1.84; ●, VL of A1.17.

*Role of the Light Chain in Cardiolipin Binding*. Analysis of VH and VL sequences of antibodies A1.72 and A1.84 showed that they had identical VL sequences despite different VH sequences (Figure 2, panels A and B). It seemed unlikely that the identical sequence would be derived independently from different VL genes by somatic mutation, rather, this VL sequence represents a germ-line sequence with limited somatic mutations. We amplified and sequenced the germ-line unrearranged gene from liver and found identical with the VL sequence of the germ-line gene Vk21E, derived from a different mouse strain (*26*). There were four nucleotide substitutions resulting in two amino acid changes in the CDR regions (valine$^{51}$ for alanine$^{51}$ in CDR2 and isoleucine$^{91}$ for serine$^{91}$ in CDR3) and two amino acid changes in the framework region, (aspargine$^{36}$ for tyrosine$^{36}$ and arginine$^{45}$ for lysine$^{45}$ (Figure 2c). A monoclonal anticardiolipin antibody containing the same V gene (Vk21E) and Jk gene (Jk2) has been described before in murine lupus (*27*), a finding consistent with the notion that lupus autoantibodies make use of restricted V gene sequences. We hypothesized that this common light-chain sequence was a major determinant of the antibody interaction with cardiolipin and tested this hypothesis by shuffling the common VL sequence (VL 72/84) with VH sequences of other antibodies. When VL 72/84 was combined with the VH sequence of a monoclonal antibody to vitronectin, H3.5, or an antibody against $\beta$2-glycoprotein I, A1.17, the resulting scFvs (VH 3.5 + VL 72/84 or VH 1.17 + VL 72/84) had reactivity toward cardiolipin (Figure 3A). In contrast, neither VH A1.72 nor VH A1.84 imparted this activity when combined

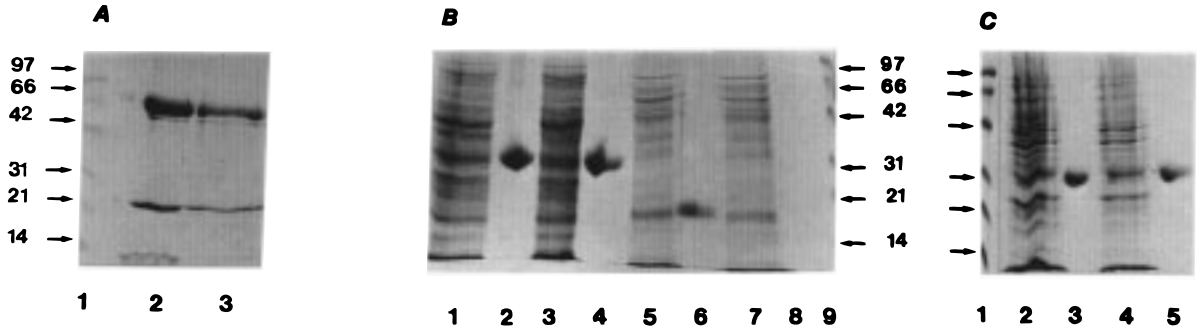



FIGURE 4: Immunoaffinity purification of scFvs, light chains, and intact IgM: hybridoma culture supernatants or periplasmic lysates of bacteria synthesizing the antibody fragments were incubated with cardiolipin liposomes, washed, and the liposome bound antibodies were eluted with 1 M NaI. The purified antibodies (30 mg) and the fragments were analyzed by SDS−PAGE in 10% gels under reducing conditions. (A) Hybridoma-derived antibodies. Lane 1, molecular weight standard; lane 2, purified A1.72; and lane 3, purified A1.84; (B) Recombinant fragments from periplasmic extracts: lanes 1, 3, 5, and 7, periplasmic extracts of A1.72, A1.84, A1.72/84 light chain and control A1.17 light chain; lanes 2 and 4, immunoaffinity purified recombinant scFvs of A1.72 and A1.84; lane 6, immunoaffinity purified A1.72/84 light chain; and lane 8, control light chain extracts subjected to immunoaffinity purification. (C) Lane 1, molecular weight standard; lanes 2 and 4, periplasmic extracts of scfv of VH3.5 + VL 72/84 and VHA1.17 + VL 72/84. Lanes 3 and 5 immunoaffinity purified scfv of VH 3.5 + VL 72/84 and VH A1.17 + VL 72/84.

with VL A1.17. These results show that the VL 72/84 alone carries all of the sequence required for cardiolipin binding.

*Light Chain Alone Interacts with Cardiolipin*. When the VL 72/84 sequence was expressed alone in bacteria, without the heavy chain, and tested for cardiolipin-binding activity, it exhibited dose-dependent binding to cardiolipin, behaving similarly to intact A1.72 and A1.84. In contrast, the VL sequence from A1.17, a monoclonal reacting with $\beta$2-glycoprotein I, did not exhibit cardiolipin binding (Figure 3B). These surprising results indicate that the light chain alone, independent of the presence of the heavy chain, exhibits specific binding affinity for cardiolipin.

*The Dissociation Constant of Light Chain Is Similar to That of the Whole Antibody*. Bacterially expressed scFvs and light chains were isolated to homogeneity by adsorption onto cardiolipin liposomes (*27*). Similarly the intact IgM was isolated from tissue culture fluids. When analyzed in SDS−PAGE under reducing conditions (Figure 4), the scFvs and the light chain had a molecular size of 34 and 17 kDa, respectively. Similar molecular size proteins were also detected in immunoblots using goat antibody to mouse $\kappa$ chain (Figure 5). Some proteolytic degradation products were detected in scFv preparations. The monoclonal antibodies exhibited heavy chains of 60 kDa and light chains of 20 kDa. The dissociation constants of the purified antibody A1.72, A1.84, recombinant scFv fragments, and the light chain were determined by an ELISA method and analyzed by Scatchard plot, as described previously (*22, 23*). The dissociation constants of these preparations were virtually identical: 21 ± 0.01 nM for the light chain VL72/84, 18.7 ± 0.09 nM for scFv of A1.72, 23 ± 0.04 for scFv of A1.84, 20 ± 0.03 nM for the intact A1.72, and 22 ± 0.03 for intact A1.84 (Figure 6). When VL 72/84 was combined with the VH sequence of a monoclonal antibody to vitronectin, H3.5, or an antibody against $\beta$2-glycoprotein I, A1.17, the resulting scFvs, VH 3.5 + VL 72/84, and VH 1.17 + VL 72/84 had similar dissociation constants of 17 ± 0.007 and 18 ± 0.03 nM, respectively. These results demonstrate that the specificity and binding affinity of the two monoclonal anticardiolipin antibodies is determined entirely by the variable sequence of their common light chain.

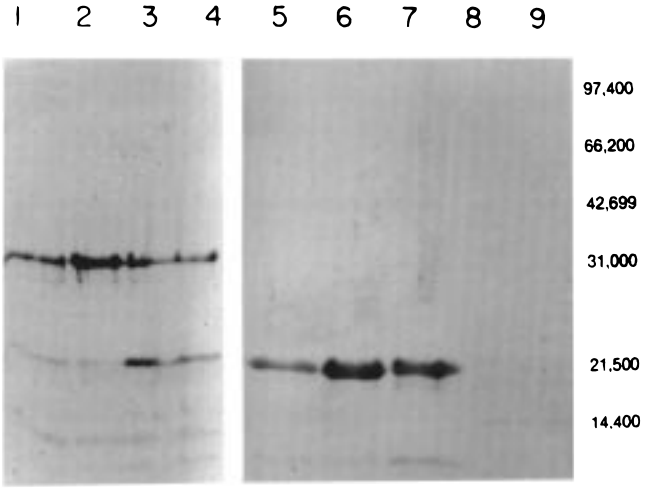



FIGURE 5: Immunoblot of scFvs and light chains. Periplasmic extracts of bacteria and immunoaffinity purified scFvs and light chain were subjected to SDS−PAGE under reducing conditions and transferred to nitrocellulose membranes. The nitrocellulose membranes were blocked and incubated with peroxidase-labeled goat antimouse $\kappa$ IgG, and the bound antibodies were detected by chemiluminescence. Lanes 1 and 2 periplasmic extracts and immunoaffinity-purified scFv of A1.72; lanes 3 and 4 periplasmic extracts and immunoaffinity-purified scFv of A1.84; lanes 5 and 6 periplasmic extracts and immunoaffinity-purified light chain VL 72/84; lanes 7 and 8 periplasmic extracts and immunoaffinity-purified control light chain VL 17; and lane 9, molecular weight markers.

## DISCUSSION

The extraordinary diversity and specificity of antibodies are due to the sequence diversity of the amino-terminal domains of their constituent heavy and light chains (*28, 29*). In each chain, the amino-terminal region contains three linear sequences of 5−10 amino acids, termed hypervariable regions or complementarity determining regions (CDR), which are held in place by more conserved sequences referred to as framework regions (*30, 31*). The CDRs form extended $\beta$ loops which are exposed on the surface of the antibody and produce a surface complementary to the three-dimensional surface of the antigen. The structural basis for these interactions has been deduced by the crystallographic analysis of a limited number of antigen−antibody complexes

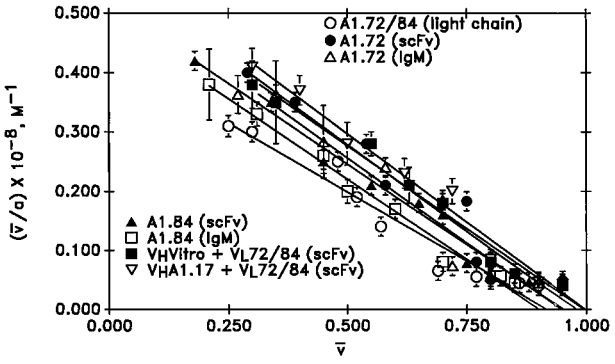



FIGURE 6: Interaction of affinity purified scFvs, light chain, and intact IgM with cardiolipin: dissociation constants for the antibodies and their fragments were determined by ELISA, as described previously (Friguet *et al.*, 1985). ○, VL of A1.72/1.84; ●, scFv of A1.72; △, intact A1.72 IgM; ▲, scFv of A1.84; □, intact A1.84 IgM; ■, scFv of VH 3.5 + VL 72/84; ▽, scFv of VH A1.17 + VL 72/84.

(*32*, *33*). In general, the heavy chain is thought to be the predominant contributor to the free energy of binding, and the contribution of the light chain to antigen binding is limited (*34*). Analyzing a large database of amino acid sequences, Kabat and Wu showed that many antibodies of different specifities assemble identical light chains with different heavy chains, but rarely the same light-chain sequences were seen in two antibodies of the same specificity (*35*). Heavy chains alone have been shown to interact with a variety of antigens in a specific manner (albeit with lower affinity than the intact antibodies), which has led to the use of single domain antibodies derived from the heavy chain (*36*). The heavy-chain dominance in binding of DNA by humam and murine autoantibodies has been shown before (*37*−*39*).

Antigen binding by light chains has been demonstrated occasionally (*40*). It has been shown among human IgM $\kappa$ rheumatoid factors the light-chain sequence derived from a germ-line $\kappa$ gene determines the binding (*6*). We show here a single light-chain variable sequence contains all the determinants necessary for cardiolipin binding with an affinity similar to that of intact antibody. This sequence is seen in two independent anticardiolipin antibodies, and these sequences differ in only four positions from that of a germ-line sequence, which belongs to VK21E [Heinrich *et al.*, (*26*)]. The same VL and Vk sequence was noted previously in a monoclonal anticardiolipin antibody derived from F1 male NZW × BXSB (*27*). It is possible that this gene is a precursor of more diversified antibodies that react with cardiolipin. The CDR-1 domain of this light chain is relatively large and has a net positive charge while CDR-2 and CDR-3 are relatively neutral, a structural feature that may play a role in the reactivity with cardiolipin.

The diversity of the CDR is generated by somatic mutations of the germ-line-coded genetic information during antigen-driven antibody maturation (*28*). Many autoantibodies have been demonstrated to undergo limited number such antigen-driven somatic mutations, and many are totally germ-line-coded (*4*−*8*). Our finding that a light-chain sequence alone determines cardiolipin binding and the fact that this sequence is close to the germ-line configurations may explain the wide occurrence of these antibodies in a variety of disorders associated with increased antibody production and raises the possibility that antigen specificity of certain antibodies may exclusively reside in their light-chain sequences.

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