# Lupus-specific Antibodies Reveal an Altered Pattern of Somatic Mutation

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## Abstract

The F4 idiotype is a heavy chain determinant expressed almost exclusively on IgG immunoglobulins and is highly associated with specificity for double-stranded DNA. Since high-titered F4 expression is present predominantly in sera of patients with systemic lupus erythematosus (SLE), we thought F4<sup>+</sup> IgG antibodies might constitute a useful subset of immunoglobulins in which to investigate lupus-specific alterations in variable (V) region gene expression or in the process of somatic mutation. This molecular analysis of F4<sup>+</sup> B cell lines generated from lupus patients demonstrates that despite the strong association of F4 reactivity with specificity for native DNA, there is no apparent V<sub>H</sub> gene restriction. Furthermore, V<sub>H</sub> gene segments encoding these antibodies are also used in protective immune responses.

An examination of the process of somatic mutation in F4<sup>+</sup> antibodies showed no abnormality in frequency of somatic mutation nor in the distribution of mutations in complementarity-determining regions or framework regions. However, there was a decrease in targeting of mutations to putative mutational hot spots. This subtle difference in mutations present in these antibodies may reflect an intrinsic defect in mutational machinery or, more likely, altered state of B cell activation that affects the mutational process and perhaps also negative selection. (*J. Clin. Invest.* 1997. 100:2538–2546.) Key words: autoreactivity  $\cdot$  anti–double-stranded DNA antibody  $\cdot$  idiotype  $\cdot$  mutational hot spots  $\cdot$  variable region gene expression

### Introduction

Idiotypic analyses have been useful in revealing the molecular genetics of specific antibody responses. In particular, idiotypic analyses of anti-DNA antibodies have helped reveal the structural and genetic heterogeneity that characterizes this pathogenic autospecificity. Data from several laboratories demonstrate that most idiotypic specificities present on anti-DNA antibodies are also present on normal serum Igs (1). This observation implies the existence of a structural and genetic relationship between autoantibodies and antibodies that are pre-

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© The American Society for Clinical Investigation, Inc. 0021-9738/97/11/2538/09 \$2.00 Volume 100, Number 10, November 1997, 2538–2546 http://www.jci.org sumably part of a protective immune response. However, a few idiotypes are highly restricted to DNA-binding antibodies and to individuals with SLE (2, 3). Analysis of the molecular genetics of these idiotypes may provide clues to SLE-specific Ig expression.

We have identified three lupus-associated anti-DNA idiotypes. 3I and 8.12 are expressed in high titer in lupus sera, yet are also present on antipneumococcal antibodies from nonautoimmune individuals (4, 5). The 3I idiotype is present on  $\kappa$ light chains, primarily those encoded by variable (V)<sup>1</sup> region  $\kappa$ I genes (6), while the 8.12 idiotype is encoded exclusively by  $V\lambda II$  light chain genes (7). Both idiotypic determinants can be encoded by germ line genes without somatic mutation, although all anti-DNA antibodies expressing these idiotypes display evidence of somatic mutation. We have interpreted these observations to mean that 3I<sup>+</sup> and 8.12<sup>+</sup> antibodies can be generated routinely in response to bacterial antigens; specificity for DNA in these idiotypic systems may be generated somatically, and a defect in the regulation of autoreactive B cells in individuals with SLE may permit the survival of cells acquiring anti-DNA specificity.

The F4 idiotype is an anti-DNA-associated idiotype that is not present in the repertoire of nonautoimmune individuals or on antipneumococcal antibodies. It is present in high titer in  $\sim 50\%$  of lupus patients with anti-double-stranded (ds) DNA activity, and from 30 to 50% of anti-DNA antibodies are F4 reactive (3). The F4 determinant is present on the heavy chain variable region, and is expressed almost exclusively on IgG antibodies (3). These observations have led us to suggest that both the F4 idiotype and DNA binding are acquired by somatic mutation, and that the F4 idiotype is intimately related to DNA specificity. As high-titered expression of this idiotype is present in SLE, a molecular genetic characterization of F4<sup>+</sup> antibodies might reveal differences in Ig variable region gene expression or in the process of somatic mutation in lupus patients that might predispose them to autoantibody production.

In this study, we characterize 10 F4<sup>+</sup> mAbs, 8 of which are novel, and 2 of which have been reported previously (6). The molecular genetic analysis of these antibodies confirms previous serologic studies, demonstrating a strong association of the F4 idiotype with IgG isotype and with DNA binding. F4 heavy chains are encoded by  $V_H$  genes that are used in protective antibody responses. A comparison of somatic mutation in these antibodies and in antibodies from nonautoimmune individuals encoded by the same  $V_H$  genes shows a subtle alteration in the pattern of mutation in SLE antibodies. Somatic mutation in the SLE  $V_H$  gene is targeted to a lesser extent to known mutational hot spots, suggesting an intrinsic B cell defect or an alteration in B cell activation in SLE.

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<sup>1.</sup> Abbreviations used in this paper: CDR, complementarity-determining region; ds, double-stranded; FR, framework region; R, replacement; S, silent; Sm, Smith antigen; V, variable (region);  $V_H$ , heavy chain variable region;  $V_L$ , light chain variable region.

Table I. F4<sup>+</sup> EBV-transformed Cell Lines

Patient	Line	Isotype	31	$V_{\rm H}$	$\boldsymbol{J}_{\mathrm{H}}$	$V_L$	$J_L$
A*	78	γ, κ	+	3	5	к2	2
$\mathbf{B}^{\ddagger}$	5–3	γ, κ	+	3	4	к3	1
$\mathbf{B}^{\ddagger}$	12-2	γ, κ	+	3	6	к2	4
$\mathbf{B}^{\ddagger}$	17–3	γ, λ	_	3	4	λ3	λ2/3
H§	17	γ, λ	_	3	4	λ3	λ2/3
M*	36	γ, κ	+	1	3	к1	2
MC <sup>∥</sup>	90	γ, κ	+	3	4	к1	2
O*	50	γ, λ	_	4	3	λ3	λ2/3
D¶	I–2a	γ, κ	+	3	4	к1	4
$H^{*\ddagger}$	H2F	γ, к	+	3	4	к4	1

Characteristics of F4<sup>+</sup> EBV lines denoting patient origin, isotype, 3I reactivity, and  $V_H J_H$  and  $V_L J_L$  usage. Patient clinical status at time of blood drawing: \*renal disease; <sup>‡</sup>arthritis; <sup>§</sup>sample was obtained 4 yr later than H2F, when patient had only arthritis; <sup>∥</sup>asymptomatic; <sup>¶</sup>thrombocytopenia.

#### Methods

#### Generation of F4<sup>+</sup> B cell clones

Peripheral blood from six individuals (designated A, B, H, M, MC, and O) with SLE (as defined by the American College of Rheumatology criteria) was obtained by venepuncture. At the time of blood collection, patients A, O, and M had active renal disease, B and H had arthritis only, and MC had no clinical symptomatology. All patients but O had a history of elevated anti-DNA titers. Four patients, B, H, M, and MC, also had elevated F4 reactivity. A seventh patient, D, underwent splenectomy for clinical indications; therefore, splenocytes were used (6). No serum was available from this patient. Lymphocytes were obtained by Ficoll-Hypaque separation of whole blood and transformed using EBV as described previously (6, 8).

#### Assays for isotype and for F4 idiotype

Idiotypic reactivity was tested as follows. ELISA wells were coated with purified mouse F4 mAb at 20  $\mu$ g/ml and incubated at 37°C for 1 h. After blocking (3% BSA in PBS), culture supernatants were added for 1 h at 37°C followed by peroxidase-conjugated goat anti-

Table II. Millipore Filter Assay for Binding to dsDNA

Cell line	Mean±SD	dsDNA reactivity
	cpm	
A78	45±14.4	_
B5-3	86±25.2	_
B12-2	$245 \pm 59.9$	+
B17-3	$75 \pm 21.8$	_
H17	$640 \pm 41.9$	++
M36	396±36.8	+
MC90	410±15.2	+
050	$3047 \pm 143.6$	+ + +
Human Ig	$21\pm0$	_

Supernatants from EBV cell lines (2  $\mu$ g/ml) and human Ig (5  $\mu$ g) were incubated with <sup>32</sup>P-labeled dsDNA and passed through a nitrocellulose filter. Reactivity is scored as follows: – (0–100 cpm), + (100–500 cpm), ++ (500–1,000 cpm), and +++ (> 1,000 cpm).

Table III. Comparison of Rearranged V Regions with Germ Line Genes

Clone	$\mathbf{V}_{\mathrm{H}}$	Germ line*	NA‡	AA§	$V_{\rm L}$	Germ line*	NA‡	AA§
A78	3	DP35	93.9	87.8	к2	A2	87.6	83.2
B5–3	3	DP49	94.2	88.8	к3	A27	96.5	92.6
B12–2	3	DP51	90.1	86.7	к2	A2	86.3	83.2
B17–3 <sup>∥</sup>	3	DP47	94.5	86.6	λ3	IGLV3S2	96.2	96.5
H17 <sup>∥</sup>	3	DP47	95.9	90.8	λ3	III.1	95.3	94.2
M36	1	hv1263	89.5	82.7	к1	08	96.4	90.5
MC90	3	DP58	96.3	94.9	к1	08	98.0	96.8
050∥	4	71–4	96.2	89.7	λ3	hlv318	96.9	96.5
I−2a¶	3	hv3005-f3	93.4	90.8	к1	L8	95.0	91.6
H2F <sup>¶</sup>	3	DP47	89.8	80.6	к4	B3	98.0	93.1

Summary of germ line gene usage by  $F4^+$  EBV-transformed lines. \*References 10–13. \*Percentage of nucleic acid (*NA*) homology. \*Percentage of amino acid (*AA*) homology. #Homologies for these lines only reflect sequence beginning at codon 10. \*Reference 4.

human IgG/IgM/IgA (Organon Teknika-Cappel, West Chester, PA). ABTS peroxidase substrate was added (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). The OD was read at 405 nm on an automated ELISA reader. 3I and 8.12 reactivity were analyzed similarly, using purified 3I or 8.12 antibody adsorbed to ELISA plates.

Cells from wells containing  $F4^+$  antibody in the supernatant were cloned by limiting dilution as described previously (6), and retested for expression of the F4, 3I, and 8.12 idiotypes as described above.

Culture supernatants of F4<sup>+</sup> clones were tested for the presence of human  $\kappa$  or  $\lambda$  and  $\mu$ ,  $\gamma$ , or  $\alpha$  chains in a standard ELISA. Briefly, polyclonal goat anti–human IgM/IgG/IgA (heavy and light) antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) was adsorbed to ELISA plates (Falcon 3915; Becton Dickinson Labware, Lincoln Park, NJ) for 1 h at 37°C. After blocking, culture supernatants were added for 1 h at 37°C, followed by peroxidase-conjugated goat anti–human IgM/IgA/IgG  $\kappa$  or  $\lambda$  (1:1,000) (Southern Biotechnology Associates, Inc.) for 1 h at 37°C. ABTS peroxidase substrate was added, and the OD was read at 405 nm using an ELISA reader (Titertek Multiscan Plus; Ealabs, Finland).

#### Antigen specificity

*dsDNA binding assay.* Binding to dsDNA was determined by Millipore filter assay (Millipore Corp., Bedford, MA) (6). Briefly, supernatants were normalized for Ig concentration and tested for binding to plasmid dsDNA using nick-translated <sup>32</sup>P-labeled DNA made double-stranded previously by passage through a nitrocellulose filter (HAWP 45; Millipore Corp.). Supernatants were not tested for single-stranded DNA binding.

Reactivity to Smith antigen (Sm), cardiolipin, and pneumococcal polysaccharide. Direct ELISAs were performed to test for other autoantigenic specificities according to a modified protocol from Swanson et al. (9). Briefly, wells were coated with Sm (10  $\mu$ g/ml) overnight at 4°C. Supernatants (5  $\mu$ g/ml) were added for 45 min at 25°C. Bound samples were detected with a goat anti–human Ig (heavy and light) alkaline phosphatase–labeled conjugate (Southern Biotechnology Associates, Inc.) and developed using 104 substrate (Sigma Chemical Co., St. Louis, MO). Binding of cardiolipin (Fluka Chemical Co., Ronkonkoma, NY) was tested by adsorbing cardiolipin, 50  $\mu$ g/ml in ethanol, to Immulon II microtiter plates overnight (Dynatech Laboratories, Inc., Chantilly, VA). Samples were added for 2 h at 25°C, and bound antibody was detected as described above.

To assay binding to pneumococcal polysaccharide, 4  $\mu$ g Pneumovax<sup>®</sup> 23 (Merck Sharp and Dohme, West Point, PA) was coated

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on ELISA immunoadsorbant assay/RIA high-binding plates (Costar Corp., Cambridge, MA) overnight at 4°C. After washing with PBS-Tween (0.05%) and blocking (3% BSA in PBS), supernatants were added for 1 h at 37°C, and the assay proceeded as described above.

#### Total RNA preparation

Total RNA was prepared from cell lines using guanidinium thiocyanate (6) or the Snap-O-Sol nucleic acid preparation kit (Biotecx Laboratories, Houston, TX). Northern blot analysis (6) was used to verify

A78 DP35	CCG GGG AAG GGG CTG GAG TGG GTT TCA TAC ATT AGT AAT TGT AGT CGT TAC ACA AAC TAC G A G A AC T T	H17 DP47	CCA GGG AAG GGG CTG GAG TGG GTC CCA ÄGA ATT AGT GGT CGT GGT AGC ACA TAC TAC T GCT A
A78 DP35	GCA GAC TCT GTG ANG GGC CGC TTC ACC ATC TCC AGA GAC AAC GCC AAC AAC TCA CTG TTT A G G A	H17 DP47	GCA GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AAA GAC AAT TCC AAG AAC ACG CTG TAT G
A78 DP35	ctg can atg arc age ctg aga gec gag gac geg gec gtg tat gac tgt geg aga gag gag gag $\overset{_{10}}{a}$	H17 DP47	ctg can atg arc age ctg aga gec gac gec gec gec gta tat tat tec tgt geg teg agt $\overset{\otimes}{g}$ a g $^{\otimes}$ a g
A78 DP35	gat att gta gta gta cca act gag ggt gac gtt gcc tcg agg cgg act ggc tog ttc gac $J_{\mu}{\rm 5}$	H17 DP47	agt GCC tat tac GGT att atg act cat tat tat agc titt gac tac tgg GGc cag GGa acc $J_{\mu}{}^{\rm d}$
A78	CCC TOO GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA	H17	CTG GTC ACC GTC TCA
		M36 hv1263	can ste can ets geg cas tet ses set ${\rm Gas}$ geg and ans and cet ses tee sea and ${\rm Geg}$
B5-3 DP49	cag gts cag ctg gts gas tet ggs gga dec gts gte ctc cag eet ggg aag tee etg aga etc $\overset{iii}{\underset{g}{\overset{ii}{}{{{{{}{}$	M36 hv1263	TCC TGC ANG GCT TCC GGA GGC ACC TTC ANG ANG TATC GTC ANG TGTC ANG ATG CGG CAG GCC T G G A G G A G G A
B5-3 DP49	TEC TOT GCA GCC TET GGA TTC ACC TEC AGT TC AGT TGGC ATG GAC TGG GTC CGC CAG GCT T	M36 hv1263	$\begin{array}{c c c c c c c c c c c c c c c c c c c $
B5-3 DP49	$\begin{array}{c} & \\ \begin{array}{c} & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & $	M36 hv1263	             
B5-3 DP49	GCA GAC TCC GTG ANG GGC CAN TTC AGC ATC TCC AGA GAC AAT TCC ANG AAC ACG CTG TAT G C	M36 hv1263	atg gaa ctg age age ctg aga tet gaa $\stackrel{50}{\text{G}}$ acg gee gtg tat tae tgt geg aga cga aag $\overset{60}{\text{G}}$
B5-3 DP49	ctg can atg and agc ctg aga gct gag gac acg gct atg tat ttc tgt gcg aga gat cat g $\overset{\approx}{\mbox{gac}}$	M36 hv1263	ang gag tug ctg ata ccc ggg gct titt gat ctc tog ggc can ggg aca atg gtc acc gtc $J_{\mu 3}$
B5-3	cca gct ggt act ggg agc ttt gac tcc tgg ggc cag gga acc ctg gtc ${\rm J}_{g4}$	M36	TCT TCA
B12-2 DP51	GAG GTG CTT CTG GTG GAG TCT GGG GGA GGC CTG GTC AAG CCT GGG GGG TCC CTG AGA CTC AG CTT A C	MC90 DP58	GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGA GGG TCC CTG AGA CTC
B12-2 DP51	$\begin{array}{c c} & & & \\ & & & \\ \hline \  \  \  \  \  \  \  \  \  \  \  \  \$	MC90 DP58	TCC TGT GGA GCC TCT GGA TTC ACC TTT AGT AGT TAT GAA ATG AAC TGG GTC CGC CAG GCT C C C
B12-2 DP51	$\begin{array}{c} \begin{array}{c} & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & $	MC90 DP58	CCA GOG ANG GGG CTG GAG TGG CTT TCA TAC ATT AGT CGT AGT GGT GAT ACC ATA TAC TAT G A AG C
B12-2 DP51	GCA GAC TCA GTG AAG GGC CGA TTC ACC GTT TCC AGA GAC CAC GCC AAG AAC TCA CTG TAT T A C A T	MC90 DP58	GCA GAC TET GTG AAG GGE CGA TTE ACE ATE TEE AGA GAE AAE GEE AAG AAE TEA ETE A
B12-2 DP51	ctg car atg gac agc ctg aga gcc gag gac acg gct gtc tat tac tgt gcg aga tcc cca $\stackrel{_{10}}{A}$ $\stackrel{_{20}}{A}$ $\stackrel{_{20}}{G}$	MC90 DP58	ctg can atg arc agc ctg aga goc gag gac acg cet gtt tat tac tgt gog aga ggc ccc T $_{\rm T}^{\rm 20}$
B12-2 DP51	CTT TGC ACT TAC GAC TGC CAC TAC TAT CGT ATG GAC GTC TGG OGC CAA GGG ACC ACG GTC $J_{\pi^6}$	MC90 DP58	cgc tac gaa ggg ccg tac tac tac tTT gac tac tgg ggc cag gga acg cTg gTc acc gTc ${\rm J}_{\rm X}4$
B12-2	ACC GTC TCC TCA	MC90 DP58	TCC TCA
B17-3 DP47	gag gtg cag ctg tta gag tet ggg gga $\mathop{\rm Ggc}\limits^{10}$ ttg gta cag cet ggg ggg tec ttg aga ctc $\mathop{\rm Ggg}\limits^{20}$		
B17-3 DP47	$\begin{array}{c c} \hline \\ \hline \\ \hline \\ \\ \hline \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ $	050 V71-4	CAG GTG CAG CTG CAG GAG TOG GGC CCA GGA CTG GTG AAG CCT TOG GAG ACC CTG TCC CTC
B17-3 DP47	$\begin{array}{c} \\ & \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ $	050 V71-4	ACC TGC ACT GTC TCT AGT GGC TCC ATC AGT AGT CAC TAC TGG AAC TGG ATC CGG CAG ACC G G T G CC I CTP2
B17-3 DP47	GCA GGC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAT TCC AAG AGC ACG TTG TAT $A$	050 V71-4	CCA GGG AAG GGG CTG GAA TGG ATT GGG AAT ATC TAT TAC AGT GGG AGC ACC AAC TAC AAC A G T
B17-3 DP47	ctg car atg arc agc ctg aga gcc gag gac acg gcc ctr tat tac tgt gcg agg gat tct g $\stackrel{_{10}}{}$ ar $\stackrel{_{20}}{}$	050 V71-4	CCC TCC CTC AAG AGT CGA GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG TTC TCC CTG C
B17-3 DP47	tig tig gan tat agt tat ogt ccc ang anc tit gac ctc tig ggc cag gga acg ctg gtc $J_{\rm J} 4$	050 V71-4	ang gtg age tet gtg ace tet geg gae acg gee gee gee tet tae tet geg aga gag gea gea $\overset{78}{\text{c}}$
B17-3	ACC	050	Get GGT tat gat get tit gat ate tog gge caa ggg aca atg gte ace gte tet tea $J_{\rm g3}^{-3}$

H17 DP47	TCC	TGT	GCA	GCC	TCT	GGA	TTC	ACC	TTT	ÅGC	AGC	TAT	GCC	ATG	ACC G	TGG	GTC	CGC	CAG	GCT
										1									CDR	2
H17 DP47	CCA	GGG	AAG	GGG	CTG	GAG	TGG	GTC	CCA T	AGA GCT	ATT	AGT	GGT	CGT A	GGT	GGT	AGC	ACA	TAC	TAC
H17 DP47	GCA	GAC	TCC	GTG	AAG	GGC	CGG	TTC	ACC	70 ATC	TCC	AAA G	GAC	AAT	TCC	AAG	AAC	ACG	CTG	80 TAT
H17 DP47	CTG	CAA	ATG	AAC	AGC	CTG	AGA	GCC	GAC G	90 GAC	GCC A G	GCC	GTA	тат	TAC	TGT	GCG	CGG AAA	TCG D	AGT
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H17	CTG	GTC	ACC	GTC	TCA															
M36 hv1263	CAA G	GTC	CAA G	CTG	GTG	CAG	TCT	GGG	GCT	GAA GAA G	GTG	AAG	AAG	сст	GGG	тсс	TCG	GTA G	AAG	20 GTC
M36 hv1263	TCC	TGC	AAG	GCT	TCC T	GGA	GGC	ACC	TTC	AAC G	AAC G	TAT	GCT	GTC A	AAC G	TGG	ATG G	CGG A	CAG	dec dec
M36 hv1263	CCT	gga	CAA	GGG	CTT	GAG	TGG	ATG	GCA G	AGG	ATC	ATC	CCT	CAC AT	CTT	GAT G	GTA A	AAA GC	AAT C	ACC TA
M36 hv1263	GCA	CAG	AGG A	TTC	GAG C	GAC G	AGA	GTC	ACG	70 ATT	ACC	GCG	GAC	ACA A	TCG C	ACG	AGC	ACA	GTC C	TTC
M36 hv1263	ATG	gaa G	СТС	AGC	AGC	CTG	AGA	TCT	GAA G	ĜAC	ACG	GCC	GTG	тат	TAC	TGT	GCG	AGA	CGA D	AAG
M36 hv1263	AAG	GAG	TGG	CTG	ATA	ccc	GGG	GCT J <sub>H</sub> 3	TTT	GAT	CTC	TGG	GGC	CAA	GGG	ACA	ATG	GTC	ACC	GTC
M36	TCT	TCA																		
MC90 DP58	GAG	GTG	CAG	CTG	GTG	GAG	TCT	GGG	gga	10 GGC	TTG	GTA	CAG	ССТ	GGA	GGG	TCC	CTG	AGA	20 CTC
MC90 DP58	TCC	TGT	GGA C	GCC	TCT	GGA	TTC	ACC	TTT C	30 AGT	AGT	TAT	GAA C	ATG	AAC	TGG	GTC	cge	CAG	GCT
MC90 DP58	CCA	GGG	AAG	GGG	CTG	GAG	TGG	CTT G	TCA	i TAC	ATT	AGT	CGT A	AGT	GGT	GAT AG	ACC	ATA	TAC	TAT C
MC90 DP58	GCA	GAC	тст	GTG	AAG	GGC	CGA	TTC	ACC	70 ATC	TCC	AGA	GAC	AAC	GCC	AAG	AAC	TCA	CTG	so TTT A
MC90 DP58	CTG	CAA	ATG	AAC	AGC	CTG	AGA	GCC T	GAG	90 GAC	ACG	GCT	GTT G	TAT	TAC	TGT	GCG	AGA	GGC D	ccc
MC90 DP58	CGC	TAC	GAA	GGG	CCG	TAC	TAC	$_{J_{H}4}^{TAC}$	TTT	GAC	TAC	TGG	GGC	CAG	gga	ACG	CTG	GTC	ACC	GTC
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050 V71-4	ACC	TGC	ACT	GTC	TCT	AGT G	GGC	TCC	ATC G	ÂGT	AGT	CAC T	TAC	TGG	AAC G	TGG	ATC	CGG	CAG	ACC C R2
050 V71-4	CCA	GGG	AAG	GGG A	CTG	gaa g	TGG	ATT	GGG	AAT T	ATC	TAT	TAC	AGT	GGG	AGC	ACC	AAC	TAC	60 AAC

GAG GTT CAA CTG TTG GAG TCT GGG GGG GGC TCG GTA CAG CCT GGG GGG TCC CTG AGA CTC G G G

20 CDR1

40

H17 DP47

A A78 DP35	cag gtg cag ctg gtc gag tct ggg gga ggc ttg gtc aag cct gga ggg tcc ctg g	AGA CTC
A78 DP35	TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT GAC TTC TAC ATG AGT GGA ATC CGC A C G	cag gct
		CDR2
A78 DP35	CCG GGG ANG GGG CTG GAG TGG GTT TCA TAC ATT AGT ANT TGT AGT CGT TAC ACA G A G A AC T	AAC TAC
A78 DP35	GCA GAC TCT GTG AAG GGC CGC TTC ACC ATC TCC AGA GAC AAC GCC AAC AAC TCA $A$ $G$ $G$	CTG TTT A
A78 DP35	ctg can atg arc age ctg aga gec gag grc geg gec gtg tat gac tgt geg aga a $^{90}_{\  \   A}$	GAG GGG D
A78 DP35	gat att gta gta gta cca act gag ggt gac gtt gcc tog agg cgg act ggc t ${\rm ggg}$ ${\rm J}_{\mu}{\rm 5}$	TTC GAC

A78 B12-2 A2	ATC 1 ATC 1	rcc rcc	TGC TGC	AGG AGG A	TCT TCT	AGT AGT	CAG CAG	AGC AGC	CTC CTC	30 CTG CTG	CAT TAT C	AGT AGC T	AAT AAT G	GGA GGA	TAC TAC A G	AAC AAC C	TAT TAT	TTG TTG	GAT GAT T	TGG TGG
																I			CDR:	2
A78 B12-2 A2	TAC ( TAC (	CTG CTG	CAG CAG	AAG AAG	CCA CCA	GGG GGG C	CAG CAG	TCT TCT C	CCA CCA	50 CAG CAA G	CTC CTC	CTG CTG	ATC ATC	TAT TAT	TTG TTG GAA	GGT GGT T	TCT TCT C	TAT AAT C	CGG CGG	GCC GCC TT
A78 B12-2 A2	TCC ( TCC ( T	GGG GGG A	GTC GTC G	CCT CCT A	GAC GAC T	AGG AGG	TTC TTC	AGT AGT	GGC GGC	AGT AGT C	GGA GGA G	TCA TCA	GGC GGC G	ACA ACA	GAT GAT	TTT TTC	ACA ACA	CTG CTG	ата Ала	*0 ATC ATC
A78 B12-2 A2	AGC A AGC A C G	AGA AGA	GTG GTG	GAG GAG	GCT GCT	GAG GAG	GAT GAT	GTT GTT	GGC GGG	GTT GAT T	ТАТ ТАТ	TAC TAC	TGC TGC	ATG ATG	CAA CAA	GCT GCT AG	CTA CTA CTA A	CAA CAA G	ACC AGT CT	CCG CCG CCG
A78 B12-2 A2	TGG I CTC I -n-	J,2 ACT ACT J,4	TTT TTC	GGC GGC	CAG GGA	GGG GGG	ACC ACC	AAG AAG	CTG GTG	gag gag	ATC ATC	ААА Ала	CGA CGA							
B5-3 A27	gaa i	ACT T	GTG	TTG	ACG	CAG	тст	CCA	GGC C	ACC	CTG	TCT	TTG	тст	CCA	GGG	GAA	AGA	GCC	ACC
B5-3 A27	CTC ?	тсс	TGT C	AGG	GCC	AGT	CAG	AGT	GTT	30 AGC	AGC	AGC	TAC	TTA	GCC	TGG	TAC	CAG	CAG	40 AAA
										50		-		CDR2						60
85-3 A27 85-3	CCT	GGC	CAG	GGC CT	ccc	AGG	CTC	CTC	ATC	TAT	GGT	GCA	TCC	AGC	AGG	GCC	ACT	GGC	ATC	CCA
A27	GAC	AGG		AGI		AGI		101			GAC	CDR	3	c	ACC	AIC	 	AGA		GAG
A27		GAA	GAI		C	TG	IAI	IAC	IGI	CAG	CAG	IAI	T	G	A	CT	J,1	ACG	ne	GGC
B5-3	CAA	GGG	ACC	AAG	GTG	GAA	ATC	AAA	CGA											
B17-3 IGLV3S2	TCC	 TAT	GTG	CTG	ACT	 CAG	ĊĊĂ	 ccc	TCA	10 GTG	TCA	GTG	GCC	CCA	GGA	CAG A	ACG	GCC	AGG	ATT
	100							CDR	1	30	a.cim	ama		***		~ ~ ~	<b>ch</b> <i>c</i>	110	003	**
IGLV352	ACC	IGI	666	664	AAC	AAC	AII	GGA	T		AGI	919	CAL	166	IAC	CHG	CAG	AAG	CCM	GGC
B17-3 IGLV3S2	CAG	gcc	сст	GTA G	CTG	GTC	GTC A	TAT	GAT T	GAT	AGC	GAC	CGG	ccc	TCA	GGG	ATC	CCT	gag	cga
B17-3 IGLV352	TTC 1	TCC T	GGC	тсс	AAC	TCT	GGG	AAC	ACG	70 GCC	ACC	CTG	ACC	ATC	AGC	AGG	GTC	GAA	GCC	GGG
										80		CDR3					.1			
B17-3 IGLV3S2	GAT (	GAG	GCC	GAC	TAT	TAC	TGT	CAG	GTG	ŤGG	GAT C	AGT	AGT	AGT	GAC T	CCT A	GTG Jλ2	GTT /3	TTC	GGC
B17-3	GGA	GGG	ACC	AAG	CTG	ACC	GTC													
H17 III.1	TCC '	TAT	GAG	ĊŢĠ	ACT	CAG	 CCA	 ccc	TCA	30 GTG	TCC	GTG	TCC	CCA	GGA	CAG	ACA	GCC	AGC	20 CTC A
H17 111.1	ACC	TGT C	TCT	GGA	GAT	ААА	TTG	CDR GGG	1 GAT	30 CAA A	TAT	GCT	TCC G	I TGG	TAT	CAG	CAG	AAG	CCA	40 GGC
									t			CDR2			l					~
H17 III.1	CAG '	TCC	сст	GTG	TTG C	GTC	ATC	CAT T	CAA	GAT	CTC AG	AAG	CGG	ccc	TCA	GGG	ATC	ССТ	GAG	60 CGA
H17 III.1	TTC '	TCT	GGC	TCC	AAC	TCT	GGG	AAC	ACA	GCC	ACT	стg	ACC	ATC	AGC	GGG	ACC	CAG	GCT	atg
								I		90		с	DR3					I		
H17 III.1	GAT :	GAG	GCT	GAC	TAT	TAC	TGT	CAG	GCG	TGG	GAC	AGC	GCC AG	ACT	G A	GGG -n-	GTG $J_{\lambda}2$	GTT /3	TTC	GCC
H17 III.1	GGA	GGG	ACC	AAG	CTG	ACC	GTC	CTA												

GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC GTC ACC CCT GGA GAG CCG GCC TCC GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC GTC ACC CCT GGA GAG CCG GCC TCC

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ATC	ACT	TGC	I CAG	GCG	AGT	CAG	GAC	DR1	r ag	C AA	G A C T	AT 1	ГТА	 aat	TGG	TAT	CAG	CAA G	AAA	40 CCA
GGG	ааа	GCC	сст	AAC G	стс	CTG	ATC	TA	 GA	T GC	ат	cc i	CDR2	TTG	gaa	TCA A	GGG	GTC	CCA	fo TCA
AGG	TTC	AGT	GGA	AGT	GGA	тст	GGG	ACI	70 GA	T TI	та	CT :	FTC	ACC	ATC	AGC	AGC	CTG	CAG	eo CCT
gaa	GAC T	ATT	gca	ACC A	TAT	CAC T	TGI	CA2	a CA	G CA T	CDR TG	3 AT 1	AAT	GTC C	CCG T	TAC J,2	ACT	TTT	GGC	CAG
GGG	ACC	AAG	CTG	GAG	ATC	AAA	. CGA													
	GAC	ATC	CAG	ATG	ACC	CAG	тст	CCA	TCC	10 TCC	CTG	TC	r gc	A TO	T GT	A GG	A GAG	C AGA	GTC	ACC
				1					CDRI	L					1					
	ATC	ACT	TGC	CAG	GCG	AGT	CAG	GAC	ATT	30 AGC	AAC	TA	г тт	A AA	.T TG	g ta	T CA	g cao	ааа	40 CC#
	GGA G	ААА	GCC	сст	aag	GTC C	CTG	ATC	TAC	50 GAT	GCA	TC	CDR C AA	2 T TI	G AA G	A AC	 A GG	3 GTC	CCA	ŤC7
	AGG	TTC	AAT G	gga	AGT	GGA	тст	GGG	ACA	70 GAT	TTI	, yC.	г тт	C AC	C AI	C AG	C AG	с сто	CAG	ee CC1
	gaa	GAT	ATT	GCA	ACA	TAT	TAC	TGT	CAA	çag	TAT	CDR:	<u>з</u> гал	тст	с. с.	.  . ta t J,:	C AG	r TTI	GGC	CAC
	GGG	ACC	AAG	CTG	GAG	ATC	AAA	CGA												
.8	 TCC	 TAT	 GTG	 CTG	 ACT	 CAG	 CCA		 TCG	.10  GTG	 CAG	GTO	g gc	c cc	A GG	A CA A	g act	g GCC	AGG	20 ATI
.8	ACC	TGT	l GGG	GGA	AAC	AAC	ATT	CDR1 GGA	AGC T	30 AAA	AGT	GT	g ca	I T TG C	G TA	C CA	g ca	g aag	CCA	GGC
.8	CAG	GCC	CCT	GTA G	CTG	GTC	GTC	тат	 GAT	50 GAT	AGC	GA	82 C GG C	GCC	стс	.  NA GG	g ato	c cei	GAG	60 CGJ
.8	TTC	TCC T	GGC	TCC	AAC	TCT	GGG	AAC	ACG	70 GCC	ACC	CT	3 AC	C AI	C AG	IC AG	G GT	C GA≱	GCC	80 GGC
.8	GAT	GAG	GCC	GAC	TAT	TAC	_ tgt	CAG	GTG	30 TGG	gat	CDR:	3 FAG	T AG	T GA T A	c cc	Ι Τ GTC Jλ:	3 GT# 2/3	. TTC	GGG
	ATC GGG GAA GGA GGG GGG GGG 8 8 8 8 8	ATC ACT GGG AAA AGG TTC GAA GAC GGG ACC GGA ACC GGA ACC GGA ACC GGA ACC GGA ACC GGA ACC ACC	ATC         ACT         TGC           GGG         AAA         GCC           AGG         TTC         AGT           GGA         GAC         AAG           GGG         ACC         AAG           GGG         ACC         AAG           GGA         AAC         ATC           AGG         TTC         GGA           AGG         TTC         GGA           AGG         TTC         GGA           AGG         TCC         TAT           GGA         ACC         TGT           B         TCC         TGT           B         TCC         TGT           B         TCC         TGT           B         GAG         GCC	ATC         ACT         TGC         CAG           GGG         AAA         GCC         CCT           AGG         TTC         AGT         GGA           GGA         GAC         ATT         GCA           GGG         ACC         AAG         CTG           GGG         ACC         AAG         CTG           GGA         AAC         ACT         TGC           AGG         TTC         ACT         AGG           AGG         TTC         ACT         AGG           AGG         TCC         AAG         GGG           AGG         TCC         AAG         AGG           AGG         TCC         TGC         AAG           AGG         TCC         TGC         AAG           AGG         TCC         TGC         AGG           ACC         TGT         GGG         ACC         TGC           B         TCC         TAT         GGG         ACC         TGC           B         TCC         TAT         GGG         ACC         TGC           B         TCC         TAT         GGG         CC         ACC           B         TCC	ATC       ACT       TGC       CAG       GGG         GGG       AAA       GCC       CCT       AAC         GAG       TTC       AGT       GGA       AGT         GAA       GAC       ATT       GCA       ACC         GGG       ACC       AAG       CTG       GAA         GGG       ACC       AAG       CTG       GAA         GGA       ATC       ACC       TGC       CAG         AGG       TTC       ACT       TGC       CAG         GGA       AAA       GCC       CCT       AGG         AGG       TTC       AAA       GCC       CCT         AGG       TCC       TAT       GCA       GCA         GGG       ACC       TGT       GGG       GGA         GGG       ACC       TGT       GGG       GGA         B       TCC       TGT       GGG       GGC         B       TCC       TCC       GGC       CCT	ATC ACT TGC CAG GCG AGT GGG AAA GCC CCT AAC CTC GGG TTC AGT GGA AGT GGA GAA GAC ATT GCA ACC TAT GGG ACC AAG CTG GAG ATC GAC ATC CAG ATG ACC ATC ACT TGC CAG ATG ACC ATC ACT TGC CAG ATG ACC AGG TTC AAT GCA ACA GGA AAA GCC CCT AAG GGA GAT ATT GCA ACA GGG ACC AAG CTG GAG a trc tat did ctt ACT a ACC TGT GGG GGA AAC a CAG GCC CCT GTA CTG a TTC TCC GGC TCC AAC a GAT GAG GCC GAC TAT	ATC       ACT       TGC       CAG       GCG       AGT       CAG       TCAGT         GGG       AAA       GCC       CCT       AAC       CTC       CTG         AGG       TTC       AGT       GGA       AGT       GGA       AGT       GGA       TCT         GAA       GAC       ATT       GCA       ACC       TAT       CAA         GGG       ACC       AAG       CTG       GAA       ATC       CAG       ATC       AAA         GGA       ATC       CAG       CTG       GAG       ATC       CAG       ATC       AAA         GGA       AAA       GCC       CAG       ATG       AGG       ATC       CAG         ATC       ACT       TGC       CAG       ATG       AGG       ATC       AGG         AGG       TTC       AAA       GCC       CTG       GAA       AGT       TTC         GGA       AAA       GCC       CAAG       CTG       GAA       ATT       GAA         GAA       GAT       ATT       GCA       AAA       GCC       CTG       AAA       TTC         GGG       ACC       TAT       GTG       GTG       <	$\left  \underbrace{ \begin{array}{c} \\ \\ \end{array}} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	$  \qquad CDRI$ ATC ACT TGC CAG GCG AGT CAG GAC AT GGG AAA GCC CCT AAC CTC CTG ATC TAC GGG TTC AGT GGA AGT GGA TCT GGG ACC GAA GAC ATT GCA ACC TAT CAC TGT CAC GGG ACC AAG CTG GAG ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA ATC ACT TGC CAG ATG ACC CAG TCT CCA ATC ACT TGC CAG ATG ACC CAG TCT CCA ATC ACT TGC CAG AGT GAA TCT GGG GAA AAA GCC CCT AAG CTC TGG ATC AGG TTC AAT GGA AGT GGA TCT GGG GAA GAT ATT GCA ACA TAT TAC TGT GGG ACC AAG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG GAG ATC AAA CGA 6 TCC TAT GTG CTG GAG ATC AAA CGA 6 TCC TAT GTG CTG ACT CAG CCC 6 TCC TAT GTG CTG GAG ATC AAA CGA 6 TCC TGT GGG GGA AAC AAC ATT GGA 6 TCC TGT GGG GGA AAC AAC ATT GGA 6 CAG GCC CCT GTA CTG GTC GTC TAT 7 TC TCC GGC TCC AAC TCT GGG AAC 8 GAT GAG GCC GAC TAT TAC TGT CAG 8 GAT GAG GCC GAC TAT TAC TGT CAG	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\frac{CDR1}{G}$ ATC ACT TGC CAG GCG AGT CAG GAC ATT $\frac{1}{AGC}$ AA GGG AAA GCC CCT AAC CTC CTG ATC TAC $\frac{1}{GAT}$ GC AGG TTC AGT GGA AGT GGA TCT GGG ACA $\frac{1}{GAT}$ GT GAA GAC ATT GCA ACC TAT CAC TGT CAA $\frac{1}{CAG}$ CA GGG ACC AAG CTG GAC ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC $\frac{1}{TC}$ GGG ACC AAG CTG GAC ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC $\frac{1}{TC}$ ATC ACT TGC CAG GCG AGT CAG ACC ATT $\frac{1}{AGC}$ GGA AAA GCC CCT AAG GTC CTG ATC TAC $\frac{1}{GAT}$ AGG TTC AAT GGA AGT GGA TCT OGG ACA $\frac{1}{GAT}$ AGG TTC AAT GGA AGT GGA TCT OGG ACA $\frac{1}{GAT}$ GGA AAA GCC CTT AAG TAT TAC TGT CAA $\frac{1}{CAG}$ GGG ACC AAG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG ACT TAT TAC TGT CAA $\frac{1}{CAG}$ 8 TCC TAT GTG CTG AAC AAC ATT TGC AAA CGA 8 TCC TGT GGG GGA AAC AAC ATT GGA ACC AAA 8 TCC TGT GGG GGA AAC AAC ATT GGA ACC AAA 8 TCC TGT GGG GGA AAC AAC ATT GGA ACC AAC 8 TCC TGT GGG CGA CTC AAC TCT GGG AAC ACG 8 TCC TGT GGG CGA CAC TCT GGG AAC ACG $\frac{1}{G}$ 8 CAG GCC CCT GTA CTG GTC GTC TAT GAT $\frac{1}{G}$ 8 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 8 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 8 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 8 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 8 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC GGC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC TCC ACC TCC AAC TCT GGG AAC ACG $\frac{1}{G}$ 9 TCC TCC TCC TCC AAC TCT TCT TCT TCT TCT	TC ACT TGC CAG GCG AGT CAG GAC ATT $\stackrel{10}{AGC}$ AAG A GGG AAA GCC CCT AAC CTC CTG ATC TAC $\stackrel{10}{GAT}$ GCA T GGG ACA AGT GGA AGT GGA TCT GGG ACA $\stackrel{10}{GAT}$ GAT TT A GAA GAC ATT GCA ACC TAT CAC TGT CAA $\stackrel{10}{CAG}$ CAT G GGG ACC AAG CTG GAC ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC $\stackrel{10}{TC}$ CCC CTG ATC ACT TGC CAG ATG ACC CAG TCT CCA TCC $\stackrel{10}{TC}$ CCC CTG ATC ACT TGC CAG GCG AGT CAG GAC ATT $\stackrel{10}{AGC}$ AAC GGA AAA GCC CCT AAG GTG CTG ATC TAC $\stackrel{10}{GAT}$ GCC ACG TTC AAT GGA AGT GGA TCT AGG ACA $\stackrel{10}{GAT}$ TTT GAA GAT ATT GCA ACA TAT TAC TGT CAA $\stackrel{10}{CAG}$ TAT GGG ACC AAG CTG GAG AGT GAG ATC AAA CGA 8 TCC TAT GTG CAG AGT GAG ATC TAT TAC TGT CAA $\stackrel{10}{CAG}$ TAT GGG ACC AAG CTG GAG AGT GAA TAT TAC TGT CAA $\stackrel{10}{CAG}$ TAT GGG ACC AAG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG AAC ACA TAT TAC TGT CAA $\stackrel{10}{CAG}$ TAT GGG ACC AAG CTG GAG ATC AAA CGA 8 TCC TGT GGG GGA AAC AAC ATT GGA ACC AAA ACT 6 TCC TAT GTG CTG ATC CTG GTC TAT GAT GAT AGT 6 CAG GCC CCT GTA CTG GTC GTC TAT GAT GAT AGC 8 TCC TGT GGG CGA AAC AAC ATT GGG AAC AGG GCC ACC 7 TC TCC GGC TCC AAC TCT GGG AAC AGG GTC TGG GAT 8 TCC TCT CC GGC TCC AAC TCT GGG AAC AGG GTC ACC 8 TCC TGT GGG CGC TCC AAC TCT GGG AAC AGG GTC TGG GAT 8 TCC TCC GGC TCC AAC TCT GGG AAC AGG GTC ACC 8 TCC TGT GGG GGA TCC AAC TCT GGG AAC AGG GTC ACC 8 TCC TGT GGG CGC TCC AAC TCT GGG AAC AGG GTC ACC 8 TCC TGT GGG CGC TCC AAC TCT GGG AAC AGG GTC ACC 8 TCC TGT GGC TCC AAC TCT GGG AAC ACG GTC TAT GAT GAT AGT 8 TCC TCC GGC TCC AAC TCT GGG AAC AGG GTG TGG GAT	$\frac{CDR1}{C}$ ATC ACT TGC CAG GCG AGT CAG GAC ATT ÀGC AAG AAT C C T $\frac{1}{C}$ GGG AAA GCC CCT AAC CTC CTG ATC TAC GAT GCA TCC I GGG ACA AGT GGA AGT GGA TCT GGG ACA GAT TTT ACT I GAA GAC ATT GCA ACC TAT CAC TGT CAA CGA CAG CAT GAT I GGG ACC AAG CTG GAG ATC AAA CGA $\frac{1}{C}$ GGG ACC AAG CTG GAG ATG ACC CAG TCT CCA TCC TCC CTG TCI ATC ACT CAG ATG ACC CAG TCT CCA TCC TCC CTG TCI ATC ACT TGC CAG GCG ACT CAG GAC ATT AGC AAC TAI GGA AAA GCC CCT AAG CTG ATC TAC GAT GAT GAT GAT GAT AGC AAC TAI GGA AAA GCC CCT AAG CTC TGG ACA GAT TTT AC GGA AAA GCC CCT AAG TCT GGA ACT GAT GAT GAT GAT AGC AAC TAI GGA AAA GCC CCT AAG TCT GGA ACA GAT TTT AC GGA AAA GCC CCT AAG TCT GGA ACA GAT TTT AC GGA AAA GCC CCT AAG TGA TAT TAC TGT CAA CGA ACC TTC GGA GGA AGT GAG ATC AAA CGA $\frac{1}{C}$ $1$	$\frac{CDR1}{C}$ ATC ACT TGC CAG GCG AGT CAG GAC ATT $\stackrel{10}{AGC}$ AAG AAT TTA C T GGG AAA GCC CCT AAC CTC CTG ATC TAC $\stackrel{10}{GAT}$ GCA TCC AAT AGG TTC AGT GGA AGT GGA TCT GGG ACA $\stackrel{70}{GAT}$ TTT ACT TTC GAA GAC ATT GCA ACC TAT CAC TGT CAA $\stackrel{70}{CAG}$ CAT GAT AAT GGG ACC AAG CTG GAG ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC $\stackrel{10}{TCC}$ CC CTG TCT GC GAA AAA GCC CCT AAG CTG TCT CCA TCC $\stackrel{10}{TCC}$ CC CTG TCT GC GGA AAA GCC CCT AAG CTG GAT CAT CAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC $\stackrel{10}{TCC}$ CC CTG TCT GC GGA AAA GCC CCT AAG GTC CTG ATC TAC $\stackrel{10}{GAT}$ GC AAC TAT TT GGA AAA GCC CCT AAG GTC CTG ATC TAC $\stackrel{10}{GAT}$ GC ACC TAT $\stackrel{10}{GC}$ AGG TTC AAT GGA AGT GGA TCT GGG ACA $\stackrel{70}{GAT}$ TTT ACT TT GAA GAT ATT GCA ACA TAT TAC TGT CAA $\stackrel{70}{CAG}$ TAT GAT AA GGG ACC AAG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG GAG ATC AAA CGA 8 TCC TAT GTG CTG GAG ATC AAA CGA 6 TCC TAT GTG CTG GAG ATC AAA CGA 6 TCC TAT GTG CTG GAG ATC AAA CGA 6 TCC TAT GTG CTG GAG ATC AAA CGA 7 TC TCT GGG GGA AAC AAC ATT GGA ACC AAA AGT GTG GC 7 C CDR2 8 CAG GCC CCT GTA CTG GTC GTC TAT GAT GAT AA AGT GTG CAC C 8 TCC TOT GGG GGA AAC AAC ATT GGA ACG GTC TAT GAT GAT AA AGT GTG GC 8 TCC TGT GGG GGA AAC AAC ATT GGA ACG GTC TAT GAT GAT AAA AGT GTG GC 8 TCC TCT GGG CGA CAA TCT GGG AAC AGG GCC ACC CTG AC 8 TCC TCT GGG CGA AAC AAC ATT GGA ACC GA CCC TG GTG GC 8 TCC TCC GGC TCC AAC TCT GGG AAC AGG GCC ACC CTG AC 8 TCC TCC GGC TCC AAC TCT GGG AAC AGG GCC ACC CTG AC 8 TCC TCC GGC TCC AAC TCT GGG AAC AGG GCC ACC CTG AC 8 TCC TCC GGC TCC AAC TCT GGG AAC ACG GTG $\stackrel{10}{T}$ GC CDR3 8 GAT GAG GCC GAC TAT TAC TGT CAG GTG TGG GAT AGT AG 8 GAT GAG GCC GAC TAT TAC TCT GTC CAG GTG $\stackrel{10}{T}$ GC CDR3 8 GAT GAG GCC GAC TAT TAC TGT CAG GTG TGG GAT AGT AG	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\frac{CDR1}{CT}$ ATC ACT TGC CAG GCG AGT CAG GAC ATT ÅGC AAG AAT TTA AAT TGG C T $\frac{CDR2}{C}$ GGG AAA GCC CCT AAC CTC CTG ATC TAC GÅT GCA TCC AAT TTG GAA AGG TTC AGT GGA AGT GGA TCT GGG ACA GAT TTA ACT TTC ACC ATC $\frac{CDR3}{T}$ GGA GAC ATT GCA ACC TAT CAC TGT CGA CAG GAT GAT AAT GTC CCG GGA CA AG CTG GAO ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC TG TCT GCA TCT GT ATC ACT TGC CAG GAC ATC AAA CGA GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GT ATC ACT TGC CAG GCG AGT CAG ACC ATT AGG AAC TAT TTA AAT TG GGA AAA GCC CCT AAG GTC CTG ATC TAC GÅT GCA TCC AAT TTG AG GGA AAA GCC CCT AAG GTC TGG ATC TAC GÅT GCA TCC AAT TTG AG GGA AAA GCC CCT AAG GTC TGG ATC TAC GÅT TTT ACT TTC ACC AT GGG TTC AAT GGA AGT GGA TCT OGG ACA GÅT TTT ACT TTC ACC AT GGG ACC AAG CTG GAG AGT GGA TCT OGG ACA GÅT TAT GAT AAT CTC CC GGG ACC AAG CTG GAG ATC AAA CGA a CC TGT GGA GGA AGT GGA TCT OGG ACA GÅT TAT GAT AAT CTC CC GGG ACC AAG CTG GAG ATC AAA CGA a CC TGT GGG GGA AAC AAC TAT TAC TGT CAA CÅG TAT GAT AAT CTG TC C CC a CC TGT GGG GGA AAC AAC ATT GGA ACC AAA ACT GG GG CC CCA GG a CC TGT GGG GGA AAC AAC ATT GGA ACC AAA ACT GGT GCC CCA GC a CC TGT GGG GGA AAC AAC ATT GGA ACC GC GC ACC CTG GGG CCC TC G GGG ACC CAG CTG GAA ACA AAC ATT GGA AAC AAA ACC AT TGG TA GAT AGT GTG GCC CCA GC a CC TGT GGG GGA AAC AAC AAC ATT GGA ACC GC GC ACC CTG GGG CCC TC G GGA GCC CCT GTA CTG GTG GTC TAT GAT AGT AGC AAC GG CCC TC C CC a CC TGT GGG CGC TCC AAC TCT GGG AAC ACG GCG CCC CTG CCC ACC TC a CC GC CCC GGC CCC ACC TCT GGG AAC ACC ACC CTG GTG GAT AGT AGT ACC ACC TC a CC GC CCC GGC TCC AAC TCT GGG AAC ACG GCG CCC CCA CC a TCC TCC GGC TCC AAC TCT GGG AAC ACG GCG CCC CTG ACC ACC ACC ACC ACC ACC ACC ACC ACC AC	$  \qquad CDR1 \qquad   \qquad CDR1 \qquad   \qquad CTCC ACC TOC CAG GCC AGT CAG GAC ATT ÀCC AAG AAT TTA AAT TGG TAT C TTC ACT TTC CAG GCC ACC AGC ACC AGG CAT CAG CAT CAC ATT TTG GAA TAT C TTC AGC AAC ACC ATT CAC TGT CAG GAT GAT TTT ACT TTC ACC ATC AGC AGG TTC AGT GGA AGT GGA TCT GGG ACC ATC AGC AGC TAT CAC TGT CAA CAG CAT GAT AAT GTC CCG TAC AGC TAT GCA ACC TAT CAC TGT CAA CAG CAT GAT AAT GTC CCG TAC T J,2 GGG ACC AAG CTG GAG ATC AAA CGA GAC ATT AGC AAC TAT TGA AAT TGG TA GTC ACT TGC CAG GCC ACT AGC CAG GCC ATT TAC TTC ACA TTG AAA AGT GTG ACC CAG GCC ATT AGC AAT TTG AAAT TGG TA GGA AAT GCC CAG GCC AGT CAG GAC ATT AGC AAC TAT TTA AAT TGG TA GGA AAT GCC CAG GCC AGT CAG GAC ATT AGC AAC TAT TTA AAT TGG TA GGA AAT GCA AGT GGA AGT GAA AGT GGA ACT AAAT GCA AGC AAT AGC AAT TTG AAA AGT GGA AGT GGA ATT AGC AAC ATT TGC AAA CGA GAT TTT ACT TTC ACC ATC AGG GAA AAT GGA AGT GGA ATT AGC AAC AAT TG GAA AGA AGT GGG GCC CCA GGA CAA AAC ATT GGA AGT GGG GCC CCA GGA CAA AC ATT GGA AGT GGG GCC ACT GGG GCC CCA GGA AAT AGC ATT GGA AGT GGG GCC ACT GGG GGA AAC AAC ATT GGA AGT GGG GCC ACT GGG CCC CAA GGA CAT TTG GAT AGT GGC ACC TTG GGG GGA AAC AAC ATT GGA AGT GGG CAC CTG AGC CCA GGA CAA AC ATT GGA AGT AGA AGT GTG GCC CCA GGA CAA AC ATT GGA AGT AGT GGC ACC ATC AGC C C TG GTC GCC TTG GGG CCC CCA GGA CAA AC ATT GGA AGT AGT GGC ACC CTG AGC ACT CAC AG T TTC TCC GGC CCC TGG GTC CAA CAC ATT GGA AGT AGT GGC ACC CTG ACC ATC AGC ATC AGC ATT GGA AGT AGT GGC ACC CTG ACC ATC AGC ATC AGC ATT GGA AGT AGT GGC ACC CTG ACC ATC AGC ATC AGC ATT GGA AGT AGT GGC ACC CTG ACC ATC AGC ATC AGC ATC AGC ATT GGA AGC AAT AGT GTG GCC ACC ATC AGC ACC TTC GGG GGC CCC TCA GG AGC ACC ATC AGC AGC ATC AGC ATC AGC ATC AGC ATC AGC ACC ATC AGC ACC ATC AGC ACC TTG GGC CCC CCG GGC CCC CTG ACC ATC AGC AGC ATC AGC AGC ATC AGC ACC ATC AGC ACC ATC AGC ACC ATC AGC ACC ATC$	$  \underbrace{\text{CDEL}}_{T}   = \underbrace{\text{CDEL}$	$  \underbrace{CPR1}_{CT} = \underbrace{CPR1}_{CT} = \underbrace{CPR2}_{CT} = \underbrace{CPR2}_{T} = \underbrace{CPR2}_{$	$  \qquad \qquad$

M36 GAC ATC CAG CTG ACC CAG TCT CCA TCG TCC CTG TCT ACA TCT GTA GGG GAC AGA GTC ACC 08 A

*Figure 1.* (*A*) Nucleotide sequences of heavy chains of  $F4^+$  antibodies compared with germ line genes. These sequence data are available from EMBL/GenBank Data Libraries under accession numbers U95234 (A78), U95235 (B5-3), U95238 (B12-2), U95236 (B17-3), U95237 (H17), U95239 (M36), U95240 (MC90), and U95241 (050). (*B*) Nucleotide sequences of light chains of  $F4^+$  antibodies compared with germ line genes. These sequence data are available from EMBL/GenBank Data Libraries under accession numbers U95242 (A78), U95243 (B5-3), U95244 (B12-2), U95248 (B17-3), U95249 (H17), U95245 (M36), U95246 (MC90), and U95247 (050).

GGA GGG ACC AAG CTG ACC GTC CTA

heavy chain isotype and variable region gene family using C $\mu$  (5'-GAGGGGGAAAAAGGGTTGGGGC-3') and C $\gamma$  (5'-GCCAGG-GGGAAGACCGATGG-3') oligonucleotides and genomic DNA probes representing heavy (V<sub>H</sub>) and light (V<sub>L</sub>) chain variable gene families (6).

#### Sequence of heavy and light chain V region genes

5 µg total RNA was reverse-transcribed using a Cy oligomer and Superscript® II RNase H reverse transcriptase (GIBCO BRL, Gaithersburg, MD). Heavy chain variable region sequences were amplified with Vent polymerase (New England Biolabs Inc., Beverly, MA) by PCR using the appropriate 3' constant region oligomer in conjunction with 5' leader oligomers for V<sub>H</sub>1 (5'-ATGGACTGGACCTG-GCAGGAGTC-3'), V<sub>H</sub>3 (5'-ATGGAGTTTGGGCTGAGCTGG-3'), or V<sub>H</sub>4 (5'-ATGAAACACCTGTGGTTCTTC-3'). PCR DNA products were recovered by the Spin Bind PCR purification system (FMC BioProducts, Rockland, ME). Light chains were amplified similarly using a universal C $\lambda$  (5'-AGTGTGGCCTTGTTGGCTTG-3') or J\ 2/3 (5'-TAGGACGGTCAGCTTGGTCCCTCCGCCGAAAAC-CAC-3') or a Ck (5'-GTTCCAGATTTCAACTGCTC-3') oligomer and either 5' leader oligomers for V<sub>K</sub>I (5'-CATGAGGGTCC-CCGCTCAG-3'), V<sub>K</sub>II (5'-ATGAGGCTCCCTGCTCAGCTC-3'), or V"III (5'-ATGGAAACCCCAGCGCAGCT-3'), or a 5' framework region (FR) 1 Vλ3 (5'-TCTGTGGAGCTCCAGCCGCCCT-CAGTG-3') oligomer.

PCR products were cloned into the TA cloning vector  $pCR^{\circledast}$  II (Invitrogen Corp., San Diego, CA), while C $\lambda$  products were cloned into pBluescript II or pCR script vectors (Stratagene Inc., La Jolla, CA). All were sequenced using Sequenase version 2 (USB Biologicals, Cleveland, OH). The University of Wisconsin Genetics Computer Group was used to analyze amino acid sequences as well as to compute isoelectric points. The predicted replacement (R) to silent (S) mutation ratios were determined by a computer-generated program (rsanal) developed by Joseph Mindell, Daniel Lustgarten, and Elahna Paul (Albert Einstein College of Medicine). This program analyzes the likelihood that point mutation of each nucleotide in the V region will lead to an R or S substitution. Therefore, each nucleotide is hypothetically changed to each of the other three nucleotides, and the cumulative R/S ratio is determined.

#### Statistics

RGYW (R = A/G, Y = T/C, W = A/T) or trinucleotide hot spots were identified using a computer-generated program. The targeting of silent mutations to hot spots was determined using  $2 \times 2$  contingency tables (Statview computer program). *P* values are given for Pearson's  $\chi^2$  analysis.

## Results

Generation and characterization of F4<sup>+</sup> lines. Sera from six lupus patients were titered for dsDNA reactivity. High-titered dsDNA reactivity (greater than the mean of control sera +2SD) was observed for patients A, B, H, M, and MC (data not shown), but not for patient O. Similarly, elevated levels of F4 were observed in sera from all patients except patients O and H (data not shown). Peripheral blood lymphocytes from these patients were transformed with EBV and cloned at 1 wk, before screening for idiotype expression. In addition, splenocytes from patient D were transformed by EBV. No simultaneous serum was available for idiotypic analysis. Approximately 100 clones from each patient were screened. 21 independent idiotype-positive clones were identified, and 8 of these were maintained successfully in culture. The characteristics of these eight clones as well as two F4<sup>+</sup> antibodies (I-2a and H2F) described previously are shown in Table I. All antibodies express an  $IgG_1$  heavy chain (data not shown). This is

consistent with previous studies of myeloma proteins and serum Ig showing that F4<sup>+</sup> Igs are almost exclusively IgG (3). Seven lines express a  $\kappa$  light chain, all of which are 3I<sup>+</sup>, confirming the preferential association of F4<sup>+</sup> heavy chains with 3I<sup>+</sup> light chains (3). Three lines, each derived from a different patient, express a  $\lambda$  light chain, although none is 8.12<sup>+</sup>. This observation is also consistent with studies of myeloma proteins showing no association between F4 and 8.12 specificities.

F4<sup>+</sup> antibodies were tested for binding to dsDNA and a panel of SLE autoantigens with which anti-DNA antibodies may cross-react. Five of the eight novel F4<sup>+</sup> antibodies as well as the two antibodies reported previously (6) bound dsDNA, confirming the strong association of F4 reactivity with native DNA (Table II). None bound Sm or cardiolipin (data not shown). The antibodies were also tested for binding to pneumococcal polysaccharide, since we had shown previously that antibodies expressing anti-DNA-associated idiotypes may bind pneumococcal antigen. None was reactive to pneumococcal polysaccharide by ELISA (data not shown).

V gene usage in  $F4^+$  antibodies. Of the 10 F4<sup>+</sup> antibodies, 8 have heavy chains encoded by members of the V<sub>H</sub>3 gene family, with 3 of these deriving from the DP47 germ line gene. One antibody is encoded by a member of the V<sub>H</sub>1 gene family, and another is encoded by a gene from the V<sub>H</sub>4 family (Table III) (10–13). All V<sub>H</sub> genes have been reported to be expressed in non–DNA-binding antibodies from nonautoimmune individuals. Nucleotide sequences of the eight novel F4<sup>+</sup> variable regions are displayed in Fig. 1 A.

7 of the 10 light chains are encoded by V  $\kappa$  genes from the  $V_{\kappa}I$ ,  $V_{\kappa}II$ ,  $V_{\kappa}III$ , and  $V_{\kappa}IV$  families. The three antibodies expressing  $\lambda$  light chains all use V $\lambda3$  genes. Nucleotide sequences of novel F4<sup>+</sup> light chains are shown in Fig. 1 *B*. A FASTA computer search was used to identify the germ line genes most homologous to the genes encoding the expressed F4<sup>+</sup> lupus antibodies. Nucleic and amino acid homologies to putative germ line genes are presented in Table III.

Protein sequences of F4<sup>+</sup> antibodies shown in Figs. 2 and 3 were compared to the protein sequences encoded by the putative germ line genes. Arginines in complementarity-determining region (CDR) 3 of the heavy chain have been suggested to be an important structural motif of murine DNA-binding antibodies (14). In addition, asparagine, lysine, and glutamine have been implicated in DNA binding (15). While there was a notable absence of arginines in CDR3 of F4<sup>+</sup> heavy chains, arginine, asparagine, and charged residues were present frequently in CDR1 and CDR2, often representing an amino acid substitution from the germ line encoded sequence.

Since nephritogenic antibodies are often cationic (16–18), it was of interest to determine the isoelectric points of the heavy and light chain variable region sequences. Most F4 reactive heavy chains are cationic; only two are not, and these two derive from non–DNA-binding antibodies (Table IV). In general, the germ line encoded sequence is strongly cationic, and mutation is not required to generate the charge of the heavy or light chain variable region.

*Mutational analysis.* Extensive somatic mutation is apparent in many of the heavy chain V region sequences (Fig. 1*A*), consistent with, although not proving, the hypothesis that mutation is necessary for expression of the F4 idiotype. All light chain V region genes are also mutated somatically (Fig. 1*B*). Since the heavy chains express the SLE-specific F4 idiotype, these were analyzed extensively to see if they might provide



*Figure 2.* The deduced protein sequences of heavy chains of  $F4^+$  antibodies compared with translated germ line genes.

evidence of lupus-specific mutational processes. The R/S mutation ratios were computed over the FRs and CDRs for each variable region (Table V). Clustering of R mutations in CDRs and a higher than random R/S ratio in CDRs are both considered evidence of antigen selection. R mutations were clustered in CDRs of F4<sup>+</sup> antibodies; 70% of all R mutations were in CDRs. While the R/S ratios in CDR1 were lower than the predicted random ratios, F4<sup>+</sup> heavy chains displayed a trend for higher than random R/S ratios in CDR2.

We sought to compare the frequency of mutation in F4<sup>+</sup> heavy chain  $V_{\rm H}$  genes to that in protective antibodies encoded by the same  $V_{\rm H}$  genes derived from nonautoimmune individuals (Table VI) (19–29). No significant difference between the two groups was found; F4<sup>+</sup>  $V_{\rm H}$  genes contained 194 mutations in 10 sequences, while 437 mutations were present in 24 sequences from nonautoimmune individuals (data not shown).

Several investigators have described mutational hot spots, which appear to be targets of hypermutation (30–32). Rogozin and Kolchanov (31) have identified sequences RGYW as potential hot spots. Recently, Smith et al. analyzed the untranslated flanking regions of Ig V genes and concluded that four trinucleotide sequences, AGC, TAC, GCT, and GTA, appear to be dominant mutational hot spots (32). We were interested in determining whether these hot spots were targeted for mutation in SLE B cells. Therefore, we examined the frequency of mutation in hot spots (mutated/nonmutated) compared with the frequency of mutation in the rest of the gene in both SLE and non-SLE antibodies. To insure that the analysis was not confounded by altered selection in SLE, we analyzed only

S mutations, which could not be subject to pressures of selection. The data show that there is a significant targeting of mutation to RGYW hot spots in antibodies derived from non-SLE individuals. 34 of 1,164 hot spots were mutated; in contrast, only 64 of 5,979 non-hot spots were mutated (P < 0.0001, Table VII). This is in striking contrast to SLE antibodies, in which there was no significant targeting of mutation to RGYW motifs. 13 of 485 hot spots displayed S mutations, while 42 of 2,507 non-hot spots displayed S mutations, while 42 of 2,507 non-hot spots displayed S mutations, there again was a significant clustering of S mutations in hot spot motifs in antibodies from nonautoimmune individuals (P < 0.0001, Table VII). In SLE antibodies, targeting to trinucleotide hot spots was also evident but was less than that observed for non-SLE antibodies (P < 0.01, Table VII).

#### Discussion

The molecular genetic analysis of EBV-transformed F4<sup>+</sup> cells confirms the results of previous studies on F4<sup>+</sup> myeloma proteins and SLE sera (3). All of the F4<sup>+</sup> antibodies reported here are IgG<sub>1</sub>, confirming the observation that F4 reactivity is highly restricted to IgG. Second, there is a strong correlation between F4<sup>+</sup> expression, cationicity, and DNA binding. In fact, our results show that all but two F4<sup>+</sup> heavy chains in this study are cationic, and of the eight cationic F4<sup>+</sup> heavy chains, seven bind dsDNA. Sequence analysis revealed that the germ



*Figure 3.* The deduced protein sequences of light chains of  $F4^+$  antibodies compared with translated germ line genes.

Table IV. Isoelectric Points

Cell line	Germ line $V_{\rm H}$	Heavy chain VDJ	Germ line $V_L$	Light chain VJ
A78	9.32	6.48	7.17	5.81
B5–3	9.87	9.31	6.45	8.66
B12–2	8.07	7.79	7.17	5.04
B17–3	8.65	6.52	5.63	4.41
H17	8.65	9.28	4.36	4.51
M36	10.25	9.34	3.94	5.65
MC90	8.07	10.29	3.94	6.44
050	9.30	8.08	4.98	4.41
I–2a	9.92	8.65	8.07	8.65
H2F	8.65	8.41	7.04	11.15

line genes used to encode F4<sup>+</sup> antibodies encode cationic sequences, and that somatic mutation is not critical for their charge. Similarly, Harada et al. have found that the very cationic light chains found in anti-DNA antibodies are encoded by a  $V_{\kappa}$  gene which in its germ line configuration encodes a highly cationic sequence (17). Furthermore, an isolated heavy chain encoded by a  $V_{H}4$  variable region gene has been shown to bind dsDNA, and this heavy chain also derives from a germ line gene that is cationic (33). Antibody 050 has seven of nine CDR2 residues in common with this isolated  $V_{H}4$  heavy chain.

Since F4 is a lupus-associated idiotype (3) (60% of lupus patients have elevated titers of F4<sup>+</sup> Igs in their sera), it was possible that it would be encoded by a single V<sub>H</sub> gene. However, sequence analysis showed that several V<sub>H</sub> gene families can encode F4<sup>+</sup> heavy chains. The fact that most of the antibodies reported here are encoded by V<sub>H</sub>3 genes probably does not reflect an idiotypic bias for V<sub>H</sub>3, but rather the predominant use of V<sub>H</sub>3 genes in human peripheral blood B cells (34). Furthermore, the V<sub>H</sub> genes used to encode F4<sup>+</sup> antibodies are expressed in the repertoire of nonautoimmune individuals, suggesting that the F4 idiotype is generated by convergent patterns of somatic mutation and not by usage of a single V<sub>H</sub> gene or by an infrequently used subset of V<sub>H</sub> genes.

No cell line expresses a  $J_H1$  or  $J_H2$  gene segment. While it is tempting to speculate that the absence of  $J_H1$  and  $J_H2$  suggests

Table V. R/S Ratios in F4<sup>+</sup> V<sub>H</sub> Regions

		Actu	ial R/S	ratio		Predicted random rates*							
	FR1	CDR1	FR2	CDR2	FR3	FR1	CDR1	FR2	CDR2	FR3			
A78	0:1	1:1	1:0	7:0	4:2	2.4	12.7	2.9	3.9	3.0			
B5–3	2:0	0:1	1:0	4:0	4:0	2.4	6.2	2.9	3.4	3.1			
B12–2	4:3	0:1	1:1	5:2	4:1	2.4	10.3	2.9	4.2	3.3			
B17–3	2:1	3:0	0:0	5:0	3:1	2.5	6.2	2.9	3.1	3.1			
H17	0:2	1:0	1:0	2:0	4:0	2.5	6.2	2.9	3.1	3.1			
M36	1:5	3:0	2:1	8:1	3:3	2.6	4.4	3.1	3.2	3.2			
MC90	1:1	1:0	1:0	2:1	1:2	2.4	16.0	2.9	3.9	3.0			
O50	2:0	2:0	1:2	1:0	3:0	2.3	8.6	2.9	3.5	2.6			
I–2a	2:4	2:1	1:1	6:1	3:4	2.4	6.2	2.9	3.4	3.1			
H2F	2:4	2:1	1:1	6:1	3:4	2.5	6.2	2.9	3.1	3.1			

\*Predicted random rate was determined by a computer-generated program (rsanal) developed by Joseph Mindell, Daniel Lustgarten, and Elahna Paul (Albert Einstein College of Medicine). that receptor editing of heavy chain genes occurs frequently in individuals with SLE, studies of 34 antibodies to foreign antigens derived from nonautoimmune individuals also show that only one anti-HIV antibody (29) and one antibody-specific *Haemophilus influenzae* type B (22) are encoded by a  $J_H1$  or  $J_H2$  segment. Previous analysis of 97 autoantibodies (35) showed that only 3 antibodies are encoded by  $J_H1$  or  $J_H2$  gene segments. The absence of these gene segments appears to be a general characteristic of the human heavy chain repertoire, perhaps due to recombination signal sequences that do not favor rearrangement. Further studies of unexpressed VDJ sequences are needed to address this question.

While F4<sup>+</sup> heavy chains can associate with  $\lambda$  light chains, most F4-reactive antibodies possess k light chains, and all of these are 3I<sup>+</sup>. This confirms a previous analysis of myeloma proteins that demonstrated a significant association of F4 and 3I reactivity. We looked for indirect evidence of receptor editing by examining whether any  $V_{\kappa}$  sequences were encoded by upstream  $V_{\mu}$  segments ( $J_{\mu}$  distal) and downstream  $J_{\mu}$  segments. Three F4<sup>+</sup> antibodies (A78, M36, and MC90) use upstream V<sub>e</sub> genes; however, these do not express downstream  $J_{\kappa}$  segments. Only the B12-2 non–DNA-binding antibody expresses a  $J_{\kappa}$ distal  $V_{\kappa}$  with a downstream  $J_{\kappa}$  segment ( $J_{\kappa}4$ ). This result concurs with those of others suggesting that there is little evidence for receptor editing of light chains in these autoreactive B cells (36). Perhaps it is a lack of editing that helps predispose some individuals to the production of autoreactive B cells. In support of this argument are data showing the failure of SLE B cells to edit the A30–J<sub> $\kappa$ </sub>2–C<sub> $\kappa$ </sub> rearrangement, which is rarely expressed in nonautoimmune individuals but has been shown to exhibit nephritogenic potential in SLE individuals (37).

Table VI. Antibodies to Foreign Antigens

Antibody	Specificity	Germ line	Reference
M20003	Cytomegalovirus	hv1263	19
L08086	Rabies	hv1263	20
L08082	Rabies	DP35	20
mAb52	Rabies	DP35	20
L08088	Rabies	DP47	20
L08083	Rabies	DP47	20
L08090	Rabies	DP47	20
L25292	HIV	DP47	21
M86597	H. influenzae type b polysaccharide	DP47	22
M86601	H. influenzae type b polysaccharide	DP47	22
L14820	H. influenzae type b polysaccharide	DP47	22
L14822	H. influenzae type b polysaccharide	DP47	22
L14821	H. influenzae type b polysaccharide	DP47	22
X56526	H. influenzae type b polysaccharide	DP47	22
U27189	Cryptococcus	DP47	23
L26898	Cytomegalovirus	DP51	24
L04329	Staphylococcus A	DP51	25
L26907	Herpes	DP51	26
L38561	HIV	DP58	27
L03824	Staphylococcus A	DP58	25
L03825	Staphylococcus A	DP58	25
L03677	Hepatitis B	71–4	28
M67503	HIV	71–4	29
L08087	Rabies	71–4	20

Table VII. S	Silent M	utations	in H	lot Spots
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RGYW motif	Hot spots	Non-hot spots	
Non-SLE			
Mutated	34	64	
Unmutated	1132	5915	
Total	1164	5979 (P < 0.001)	
SLE			
Mutated	13	42	
Unmutated	472	2465	
Total	485	2507 ( $P > 0.1$ )	
Trinucleotide motifs			
Non-SLE			
Mutated	36	62	
Unmutated	1425	5622	
Total	1461	5684 (P < 0.0001)	
SLE			
Mutated	19	36	
Unmutated	579	2385	
Total	598	2421 ( <i>P</i> < 0.01)	

*P* values are determined by a  $\chi^2$  analysis of S mutations in hot spots over total hot spots, compared with S mutations in non–hot spots over total non–hot spots. This represents the targeting of mutations to hot spot motifs.

Sequence analysis confirms the hypothesis that  $F4^+$  antibodies are mutated somatically. In the heavy chain variable regions, mutations in CDR2 are more prevalent than mutations in CDR1. This is consistent with other studies showing an increased frequency of somatic mutation in CDR2 of  $V_H3$  and  $V_H4$  genes encoding autoantibodies (18, 38). Radic and Weigert have suggested that CDR2 of the heavy chain plays an important role in DNA binding, and a large number of murine anti-DNA antibodies also display extensive mutation of CDR2 in the heavy chain V region (39).

Because F4<sup>+</sup> antibodies are mutated, and because they are present almost uniquely in SLE serum, they offer the opportunity to study the pattern of somatic mutation in  $V_H$  genes used by lupus antibodies. While other SLE-associated idiotypes like 3I and 8.12 are expressed on protective antibodies in normal serum, F4<sup>+</sup> antibodies do not appear to be part of a protective response and are not present in normal serum. Therefore, we reasoned that the analysis of somatic mutation in F4<sup>+</sup> heavy chains might reveal a population of  $V_H$  genes displaying SLEspecific aspects of somatic mutation.

Analysis of R and S mutations displayed a clustering of R mutations in CDRs and a trend toward a higher than random R/S ratio in CDRs. These observations are more consistent with antigen selection than polyclonal activation, yet it is not possible from these studies to know whether the selecting antigen is a self or foreign antigen.

Smith and colleagues reported recently no difference in use of trinucleotide mutational hot spots between autoantibodies and anti-foreign antibodies in mice (32); however, they analyzed autoantibodies derived from MRL/lpr mice. MRL/lpr mice are autoimmune due to a defect in *fas* gene expression (40), which would not be expected to be involved in the process of somatic mutation. Our analysis reveals the same frequency of mutation in  $V_H$  genes encoding F4<sup>+</sup> heavy chains as in these genes encoding protective antibodies. However, we do find evidence for decreased targeting of somatic mutation to mutational hot spots when RGYW motifs are examined. When trinucleotide motifs are examined, there remains a targeting of somatic mutation to hot spots, but it is less than that seen in non-lupus heavy chains. This observation suggests that mutation can be uncoupled from targeting to hot spots. We would speculate that one complex is needed for mutation to occur, and that additional proteins are needed to target mutation to particular DNA sequences.

These results are similar to other studies in our laboratory which found that germinal center B cells of mice constitutively expressing bcl-2 show no decrease in frequency of somatic mutation of Ig genes, but a decreased targeting of mutation to putative mutational hot spots (41). Recent studies have shown that expression of bcl-2 inhibits progression through the cell cycle (42, 43). Possibly, the alteration in cell cycle kinetics leads to a change in the process of somatic mutation. More likely, constitutive expression of bcl-2 may alter gene expression in B cells and lead to an uncoupling of mutation from targeting to hot spots. Constitutive expression of bcl-2 or other alterations in B cell activation might affect expression of proteins needed to target mutation to hot spots. While the data remain inconclusive, it has been suggested that SLE B cells overexpress bcl-2 (44, 45).

It is possible, given the data presented, that there is a defect in the machinery of somatic mutation in SLE B cells, but it is more likely that the altered pattern of somatic mutation reflects a difference in B cell activation that may determine thresholds for negative selection. Alterations in B cell gene expression might affect both the targeting of somatic mutation and thresholds for negative selection. For example, increased expression of bcl-2, reported to occur in SLE B cells (44), affects both resistance to apoptosis and the targeting of somatic mutation to hot spot motifs. Alternatively, other changes in gene expression might lead to an uncoupling of mutation from targeting to hot spots. Because our analysis of mutation focuses exclusively on S mutations, we can conclude that the decreased frequency of mutation in hot spots is not a function of altered selection in SLE. We would speculate that the abnormal pattern of mutation is not itself responsible for the autospecificities that are present. Rather, we believe as stated above that overexpression of bcl-2, or some other change in B cell activation, is responsible for both the altered mutational pattern characterized by an uncoupling of mutation from targeting to hot spots, and the altered selection of B cells. We believe that SLE B cells may undergo germinal center maturation while in a different activation state. Taken together, these data might suggest that autospecificities in SLE arise due to a lack of negative selection of B cells acquiring autoreactivity through somatic mutation, rather than an antigen-specific activation of autoreactive cells. B cells in SLE may have an altered pattern of gene expression that leads to enhanced survival and an uncoupling of mutation from hot spot targeting.

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#### References

1. Isenberg, D., W. Williams, J. Axford, R. Bakimer, D. Bell, T. Casaseca-Grayson, B. Diamond, F. Ebling, B. Hahn, G. Harkiss, et al. 1990. Comparison of DNA antibody idiotypes in human sera: an international collaborative study of 19 idiotypes from 11 different laboratories. *J. Autoimmun.* 3:393–414.

2. Diamond, B., and G. Solomon. 1983. A monoclonal antibody recognizes anti-DNA antibodies in patients with systemic lupus erythematosus. *Ann. NY Acad. Sci.* 418:379–385.

3. Davidson, A., A. Smith, J. Katz, J.L. Preud'Homme, A. Solomon, and B. Diamond. 1989. A cross-reactive idiotype on anti-DNA antibodies defines a H chain determinant present almost exclusively on IgG antibodies. *J. Immunol.* 143:174–180.

4. Grayzel, A., A. Solomon, C. Aranow, and B. Diamond. 1991. Antibodies elicited by pneumococcal antigens bear an anti-DNA-associated idiotype. *J. Clin. Invest.* 87:842–846.

5. Livneh, A., E. Gazit, and B. Diamond. 1994. The preferential expression of the anti-DNA associated 8.12 idiotype in lupus is not genetically controlled. *Autoimmunity*. 18:1–6.

6. Manheimer-Lory, A., J.B. Katz, M. Pillinger, C. Ghossein, A. Smith, and B. Diamond. 1991. Molecular characteristics of antibodies bearing an anti-DNA-associated idiotype. *J. Exp. Med.* 174:1639–1652.

7. Paul, E., A. Iliev, A. Livneh, and B. Diamond. 1992. The anti-DNA-associated idiotype 8.12 is encoded by the VλII gene family and maps to the vicinity of L chain CDR1. *J. Immunol.* 149:3588–3595.

8. Davidson, A., A. Manheimer-Lory, C. Aranow, R. Peterson, N. Hannigan, and B. Diamond. 1990. Molecular characterization of a somatically mutated anti-DNA antibody bearing two systemic lupus erythematosus-related idiotypes. J. Clin. Invest. 85:1401–1409.

9. Swanson, P.C., C. Ackroyd, and G.D. Glick. 1996. Ligand recognition by anti-DNA autoantibodies. Affinity, specificity and mode of binding. *Biochemistry*. 35:1624–1633.

10. Tomlinson, I.M., G. Walter, J.D. Marks, M.B. Llewelyn, and G. Winter. 1992. The repertoire of human germline  $V_H$  reveals about fifty groups of  $V_H$  segments with different hypervariable loops. *J. Mol. Biol.* 227:776–798.

11. Cox, J.P.L., I.M. Tomlinson, and G. Winter. 1994. A directory of human germline  $V_{\kappa}$  segments reveals a strong bias in their usage. *Eur. J. Immunol.* 24: 827–836.

12. Daley, M.D., H.-Q. Peng, V. Misener, X.-Y. Liu, P.P. Chen, and K. Siminovitch. 1992. Molecular analysis of human immunoglobulin  $V\lambda$  germline genes: subgroups  $V\lambda$ III and  $V\lambda$ IV. *Mol. Immunol.* 29:1515–1518.

13. Williams, C., and G. Winter. 1993. Cloning and sequencing of human immunoglobulin Vλ gene segments. *Eur. J. Immunol.* 23:1456–1461.

14. Radic, M.Z., J. Mackle, J. Erickson, C. Mol, W.F. Anderson, and M. Weigert. 1993. Residues that mediate DNA binding of autoimmune antibodies. *J. Immunol.* 150:4966–4977.

15. Seeman, N.C., J.M. Rosenberg, and A. Rich. 1976. Sequence-specific recognition of double helical nucleic acids by proteins. *Proc. Natl. Acad. Sci. USA*. 73:804–808.

16. O'Keefe, T.L., S.K. Datta, and T. Imanishi-Kari. 1992. Cationic residues in pathogenic anti-DNA autoantibodies arise by mutations of a germ-line gene that belongs to a large  $V_{\rm H}$  gene subfamily. *Eur. J. Immunol.* 22:619–624.

17. Harada, T., N. Suzuki, Y. Mizushima, and T. Sakane. 1994. Usage of a novel class of germline Ig variable region gene for cationic anti-DNA autoantibodies in human lupus nephritis and its role for the development of the disease. *J. Immunol.* 153:4806–4815.

18. Demaison, C., P. Chastagner, J. Theze, and M. Zouali. 1994. Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype. *Proc. Natl. Acad. Sci. USA*. 91:514–518.

19. Newkirk, M.M., H. Gram, G.F. Heinrich, L. Ostberg, J.D. Capra, and R.L. Wasserman. 1988. Complete protein sequences of the variable regions of the cloned heavy and light chains of a human anti-cytomegalovirus antibody reveal a striking similarity to human monoclonal rheumatoid factors of the Wa idiotypic family. *J. Clin. Invest.* 81:1511–1518.

20. Ikematsu, H., N. Harindranath, Y. Ueki, A.L. Notkins, and P. Casali. 1993. Clonal analysis of a human antibody response. II. Sequences of the  $V_H$ genes of human IgM, IgG and IgA to rabies virus reveal a preferential utilization of  $V_H$ III segments and somatic hypermutation. *J. Immunol.* 150:1325–1337.

21. Harindranath, N., H. Ikematsu, A.L. Notkins, and P. Casali. 1993. Structure of the  $V_H$  and  $V_L$  segments of polyreactive and monoreactive human natural antibodies to HIV-1 and *Escherichia coli*  $\beta$ -galactosidase. *Int. Immunol.* 5:1523–1533.

22. Adderson, E.E., P.G. Shackelford, A. Quinn, P.M. Wilson, M.W. Cun-

ningham, R.A. Insel, and W.L. Carroll. 1993. Restricted immunoglobulin VH usage and VDJ combinations in the human response to *Haemophilus influenzae* type b capsular polysaccharide. Nucleotide sequences of monospecific anti-*Haemophilus* antibodies and polyspecific antibodies cross-reacting with self antigens. *J. Clin. Invest.* 91:2734–2743.

23. Pirofski, L., R. Lui, M. DeShaw, A.B. Kressel, and Z. Zhong. 1995. Analysis of human monoclonal antibodies elicited by vaccination with a *Cryptococcus neoformans* glucoronoxylomannan capsular polysaccharide vaccine. *Infect. Immun.* 63:3005–3014.

24. Rioux, J.D., M. Ohlin, C.A.K. Borrebaeck, and M.M. Newkirk. 1995. Molecular characterization of human monoclonal antibodies specific for the human cytomegalovirus: relationship of variable region sequence to antigen specificity and rheumatoid factor-associated idiotype expression. *Immunol. Infect. Dis.* (*Lond.*). 5:43–52.

25. Hillson, J., N.S. Karr, I.R. Oppliger, M. Mannik, and E.H. Sasso. 1993. The structural basis of germline-encoded  $V_H3$  immunoglobulin binding to staphylococcal protein A. J. Exp. Med. 178:331–336.

26. Rioux, J.D., J. Rauch, L. Silvestri, and M.M. Newkirk. 1994. A human rheumatoid factor C304 shares  $V_{\rm H}$  and  $V_{\rm L}$  gene usage with antibodies specific for human viral pathogens. *Scand. J. Immunol.* 40:350–354.

27. Pilkington, G.R., L. Duan, M. Zhu, W. Keil, and R.J. Pomerantz. 1996. Recombinant human Fab antibody fragments to HIV-1 Rev and Tat regulatory proteins: direct selection from a combinatorial phage display library. *Mol. Immunol.* 33:439–450.

28. Andris, J.S., P.H. Ehrlich, L. Ostberg, and J.D. Capra. 1992. Probing the human antibody repertoire to exogenous antigens. Characterization of the H and L chain V region gene segments from anti-hepatitis B virus antibodies. *J. Immunol.* 149:4053–4059.

29. Andris, J.S., S. Johnson, S. Zolla-Pazner, and J.D. Capra. 1991. Molecular characterization of five human anti-human immunodeficiency virus type I antibody heavy chains reveals extensive somatic mutation typical of an antigendriven immune response. *Proc. Natl. Acad. Sci. USA*. 88:7783–7787.

30. Betz, A.G., M.S. Neuberger, and C. Milstein. 1993. Discriminating intrinsic and antigen-selected mutational hotspots in immunological V genes. *Immunol. Today*. 14:405–411.

31. Rogozin, I.B., and N.A. Kolchanov. 1992. Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighbouring base sequences on mutagenesis. *Biochem. Biophys. Acta*. 1171:11–18.

32. Smith, D.S., G. Creadon, P.K. Jena, J.P. Portanova, B.L. Kotzin, and L.J. Wysocki. 1996. Di- and trinucleotide target preferences of somatic mutagenesis in normal and autoreactive B cells. *J. Immunol.* 156:2642–2652.

33. Kieber-Emmons, T., J.M. von Feldt, A.P. Godillot, D. McCallus, V. Srikantan, D.B. Weiner, and W.V. Williams. 1994. Isolated  $V_H4^+$  heavy chain variable regions bind DNA. Characterization of a recombinant antibody heavy chain library derived from patient(s) with active SLE. *Lupus*. 3:379–392.

34. Brezinschek, H.P., R.I. Brezinschek, and P.E. Lipsky. 1995. Analysis of heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. *J. Immunol.* 155:190–202.

35. Manheimer-Lory, A., R. Monhian, A. Splaver, B. Gaynor, and B. Diamond. 1995. Analysis of the  $V_{\kappa}I$  family: germline genes from an SLE patient and expressed autoantibodies. *Autoimmunity*. 20:259–265.

36. Bensimon, C., P. Chastagner, and M. Zouali. 1994. Human anti-DNA autoantibodies undergo essentially primary  $V_{\kappa}$  gene rearrangements. *EMBO* (*Eur. Mol. Biol. Organ.*) J. 13:2951–2962.

37. Suzuki, N., T. Harada, S. Mihara, and T. Sakane. 1996. Characterization of a germline  $V_{\star}$  gene encoding cationic anti-DNA antibody and role of receptor editing for development of the autoantibody in patients with systemic lupus erythematosus. *J. Clin. Invest.* 98:1843–1850.

38. Rioux, J.D., E. Zdarsky, M.M. Newkirk, and J. Rauch. 1995. Anti-DNA and anti-platelet specificities of SLE-derived autoantibodies: evidence for  $CDR2_{\rm H}$  mutations and CDR3 motifs. *Mol. Immunol.* 32:683–696.

39. Radic, M.Z., and M. Weigert. 1994. Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu. Rev. Immunol.* 12:487–520.

40. Watanabe-Fukunaga, R., C.I. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature*. 356:314–317.

41. Kuo, P., A. Alban, D. Gebhard, and B. Diamond. 1997. Overexpression of bcl-2 alters usage of mutational hot spots in germinal center B cells. *Mol. Immunol.* In press.

42. Mazel, S., D. Burtrum, and H.T. Petrie. 1996. Regulation of cell division cycle progression by bcl-2 expression: a potential mechanism for inhibition of programmed cell death. *J. Exp. Med.* 183:2219–2226.

43. O'Reilly, L.A., D.C.S. Huang, and A. Strasser. 1996. The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. *EMBO* (*Eur. Mol. Biol. Organ.*) J. 15:6979–6990.

44. Gatenby, P.A., and M. Irvine. 1994. The bcl-2 protooncogene is overexpressed in systemic lupus erythematosus. J. Autoimmun. 7:623–631.

45. Rose, L.M., D.S. Latchman, and D.A. Isenberg. 1995. Bcl-2 expression is unaltered in unfractionated peripheral blood mononuclear cells in patients with systempic lupus erythematosus. *Br. J. Rheumatol.* 34:316–320.