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Immunology

Human autoantibody recognition of DNA

(systemic lupus erythematosus/phage display/antibody libraries)

SHANA M. BARBAS*, HENRIK J. DITZEL*†, EEVA M. SALONEN‡, WEI-PING YANG§, GREGG J. SILVERMAN¶, AND DENNIS R. BURTON*§

Departments of *Immunology and §Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037; †Department of Microbiology, Odense University, 5000 Odense, Denmark; ‡Department of Virology, University of Helsinki, SF-00290 Helsinki, Finland; and ¶Department of Medicine, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0063

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ABSTRACT **Combinatorial IgG Fab phage display libraries prepared from a systemic lupus erythematosus (SLE) donor and a healthy donor were affinity selected against human placental DNA. Human monoclonal antibody Fab fragments specific for DNA were isolated from both libraries, although Fabs of the highest affinity were isolated only from the lupus library. Generally, apparent affinities of the Fabs for human placental DNA, purified double-stranded DNA, and denatured DNA were approximately equivalent. Surface plasmon resonance indicated Fab binding constants for a double-stranded oligodeoxynucleotide of 0.2–1.3 × 10^8 M^{-1}. The higher-affinity Fabs, as ranked by binding to human placental DNA or to the oligonucleotide probe, tested positive in the *Crithidia luciliae* assay commonly used in the diagnosis of SLE, and interestingly the genes encoding the heavy-chain variable regions of these antibodies displayed evidence of only minimal somatic hypermutation. The heavy chains of the SLE Fabs were characterized by a predominance of basic residues toward the N terminus of complementarity-determining region 3 (CDR3). The crucial role of heavy-chain CDR3 (HCDR3) in high-affinity DNA recognition was suggested by the creation of DNA binding in an unrelated antibody by HCDR3 transplantation from SLE antibodies. We propose that high-affinity DNA-binding antibodies can arise in SLE without extensive somatic hypermutation in the variable-region genes because of the expression of inappropriate HCDR3s.**

"Natural autoantibodies" directed against DNA are present in the serum of normal healthy individuals, are generally of low relative affinity for DNA, and exhibit polyreactivity. Such antibodies are not believed to be involved in pathogenesis of autoimmune disease (1). In contrast, high-affinity IgG antibodies specific for native double-stranded DNA (dsDNA), as detected by the *Crithidia luciliae* assay, are virtually diagnostic of the autoimmune disease systemic lupus erythematosus (SLE) (2). There is strong evidence that these antibodies identified by *in vitro* binding to dsDNA participate in the pathogenesis of SLE by depositing in the kidneys. The resulting renal damage is the leading cause of death and disability in human lupus (3). It is, however, unclear to what extent this deposition arises from *in vivo* interaction with DNA or other crossreactive antigens (reviewed in ref. 4).

We are interested in the features of antibodies that mediate DNA recognition and pathogenesis. Previous work has shown that high-affinity antibodies to dsDNA can be generated from an antibody having no significant affinity for DNA, solely by reconstruction of the heavy-chain complementarity-determining region 3 (HCDR3) (5). These experiments suggested that high-affinity binding to DNA could be dictated by HCDR3 in lupus antibodies. If so, then recombination of variable (V), diversity (D), and joining (J) gene segments might be of greater importance in generating high-affinity antibodies than somatic hypermutation.

To explore this possibility, we isolated anti-DNA Fab fragments from an SLE patient and a healthy donor by the combinatorial library approach (6). Also included in our study was an anti-DNA Fab from a human immunodeficiency virus type 1 (HIV-1)-seropositive donor (7). We reasoned that differences between the antibodies from the donors might reveal factors important for pathogenesis and would help to test the validity of the library approach in evaluation of the autoimmune situation.

MATERIALS AND METHODS

Library Construction, Selection, and Characterization of Fab Fragments. All procedures were performed essentially as described (8–11) to construct an IgG1(λ) Fab phage display library of 8 × 10^6 members from peripheral blood lymphocytes (PBLs) of a patient with active SLE. The construction of an IgG1(κλ) library of 10^7 members from a healthy donor has been described (12). Libraries were panned against human placental DNA (hpDNA) from Sigma that was dry coated onto microtiter wells (1 μg of hpDNA in phosphate-buffered saline evaporated to dryness at 37°C). DNase and RNase were added to the cultures during overnight growth to help prevent the binding of bacterial DNA debris by target Fabs. ELISAs and nucleic acid sequencing were as described (7). Comparison of Fab sequence to reported immunoglobulin germline sequences from the GenBank/EMBL data base was done with the Genetics Computer Group sequence analysis program.

Preparation of DNA. dsDNA was prepared from hpDNA by S1 nuclease treatment (13). Denatured DNA was made just prior to use by heating dsDNA at 90°C for 5 min and then diluted immediately to the working concentration in chilled phosphate-buffered saline.

***C. luciliae* Assay.** *C. luciliae* slides from Kallestad Laboratories (Chaska, MN) were used to screen the anti-DNA Fabs. Bound Fabs (purified) or antibodies from patient serum (used at 1:100 dilution) were detected with a fluorescein-labeled anti-Fab secondary antibody (Jackson ImmunoResearch).

HCDR3 Grafting Experiments. The HCDR3 sequences for Fabs SI-1, SI-40, and SI-32 were grafted onto the heavy chain of an anti-tetanus toxoid Fab, replacing the existing HCDR3, by overlap extension PCR (14, 15). Western blot analysis of HCDR3-grafted Fabs revealed colocalization of light-chain and heavy-chain bands around 50 kDa, which was interpreted as appropriately paired heavy- and light-chain heterodimers.

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Abbreviations: CDR, complementarity-determining region; HCDR, heavy-chain CDR; FR, framework region; SLE, systemic lupus erythematosus; dsDNA, double-stranded DNA; hpDNA, human placental DNA; HIV, human immunodeficiency virus; PBL, peripheral blood lymphocyte.

Surface Plasmon Resonance. Kinetic constants for the interaction of Fabs and an oligonucleotide probe were determined by surface plasmon resonance with the Pharmacia BIAcore instrument as described (16) using Fab concentrations in the range of 1–100 μg/ml and the oligonucleotide f2, which forms a duplex linked by the loop TTTT (5′-CCT-GCG-TTG-GCG-CCC-TTTT-GGG-CGC-CAA-CGC-AGG-3′).

RESULTS AND DISCUSSION

Combinatorial IgG phage display libraries were generated from PBL RNA isolated from a SLE patient and from bone marrow RNA from a healthy donor. Although bone marrow is generally preferred (6), PBLs were used for the SLE library because of tissue availability, and this appears a satisfactory source in this case, presumably due to the active nature of the disease. The libraries were panned against hpDNA which was suspended in nuclease-free water and used without further purification. Six unique clones were isolated from the SLE library (designated SI), and one positive clone was isolated from the healthy donor library (designated N).

The binding of recombinant autoimmune murine Fab fragments to denatured DNA has been described (17), but we believe that this is the first report of recombinant autoimmune human Fab fragments binding to dsDNA. Therefore we first investigated binding in terms of ELISA titrations and the *C. luciliae* assay as used in SLE diagnostic testing. As shown in Fig. 1, there was a good correlation between relative binding affinity to human placental dsDNA and *Crithidia* reactivity. Fabs exhibiting half-maximal binding at a concentration ≤1 μg/ml were clearly *Crithidia*-positive. Fab SI-39, with a concentration for half-maximal binding of 5 μg/ml, stained weakly in the assay. In a competition ELISA format, we found 50% inhibition of binding of the *Crithidia*-positive Fabs at about 1 μg/ml (data not shown), which would qualify them as high-affinity binders by the definition of Marion *et al.* (18). Fab binding to an oligonucleotide probe, consisting of 34 bases which form a 15-bp duplex linked by a $(dT)_4$ loop (16), was measured by surface plasmon resonance. Binding constants in the range 0.2–1.3 × 10^8 M^{-1} were observed (Table 1), confirming that the Fabs interacted with DNA with high affinity. The rank order of binding affinities was similar to that suggested by ELISA titration, although the range of affinities was less than suggested by ELISA. This may reflect differences in binding to a homogeneous oligonucleotide versus heterogeneous genomic DNA.

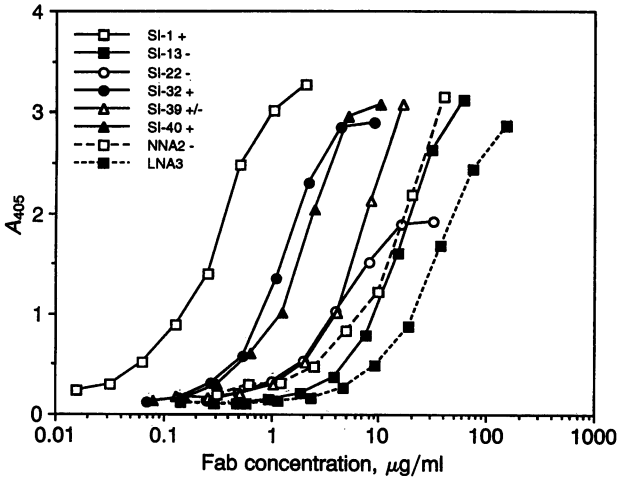

FIG. 1. Comparison of Fab binding to solid-phase human placental dsDNA by ELISA and relation to staining in the *C. luciliae* assay for purified Fabs from the SLE (SI), healthy (N), and HIV-1-seropositive (L) donor libraries. *Crithidia* staining (+ or −) is scored next to the sample name in the key.

Table 1. Kinetic constants and calculated association and dissociation constants for the binding of recombinant Fabs to an oligonucleotide duplex as measured by surface plasmon resonance

Fab	k_{on}, $M^{-1}\cdot s^{-1}$	k_{off}, s^{-1}	K_a, M^{-1}	K_d, M
SI-1	3.7×10^4	2.9×10^{-4}	1.3×10^8	7.6×10^{-9}
SI-13	2.4×10^4	6.7×10^{-4}	3.7×10^7	2.7×10^{-8}
SI-22	1.2×10^4	6.1×10^{-4}	2.0×10^7	4.9×10^{-8}
SI-32	7.0×10^4	7.8×10^{-4}	9.0×10^7	1.1×10^{-8}
SI-39	1.7×10^4	6.8×10^{-4}	2.6×10^7	3.9×10^{-8}
SI-40	3.0×10^4	3.6×10^{-4}	8.3×10^7	1.2×10^{-8}
TT(HCDR3, SI-1)	3.2×10^4	4.4×10^{-4}	7.2×10^7	1.4×10^{-8}
TT(HCDR3, SI-40)	3.4×10^4	4.5×10^{-4}	7.6×10^7	1.3×10^{-8}

TT(HCDR3, SI-1 or SI-40) indicates the Fab has the HCDR3 from Fab SI-1 or SI-40 grafted into the Fab originally binding tetanus toxoid as described in the text.

Crithidia staining by antibody is often associated with specificity for dsDNA. However, affinity is also an important consideration. Therefore, one might have predicted that staining would be dependent upon antibody concentration. In fact, *Crithidia* staining was found to be independent of the concentration of Fab used over a typical range of 1–10 μg/ml. The likely explanation is that washing removes the weaker-binding Fabs during the assay. In other words, staining is crucially dependent upon Fab off-rates, which apparently correlate with affinity for the *Crithidia* kinetoplast.

The specificity of the Fabs was further investigated by ELISA. Previously, we have shown that Fabs selected from an HIV-1-seropositive donor library by panning against human placental DNA had moderate apparent affinities for DNA, similar to the weaker binders of Fig. 1, and marked polyreactivity with a panel of antigens (7). The HIV-1 donor was originally chosen because he had a markedly elevated level of serum IgG reacting with DNA but had no symptoms of SLE disease. As illustrated in Fig. 2, crossreactivity of Fabs from the SLE donor library with the panel of antigens was low compared with a typical Fab selected from the HIV-1 donor library. However, some of the weaker DNA-binding Fabs, especially Fab SI-22, did show significant polyspecificity. The Fab (NNA2) from the healthy donor library was relatively specific for DNA.

Next we looked at the specificity of the SLE and healthy donor Fabs for denatured and duplex DNA. Most of the Fabs showed approximately equivalent binding to the two forms (Fig. 3), but one Fab (SI-22) showed a marked preference for denatured DNA. In a study of six anti-DNA IgG antibodies from SLE donors isolated by cellular methods, Winkler *et al.* (19) also reported approximately equivalent binding to denatured DNA and dsDNA.

The gene usage and amino acid sequences of the recombinant DNA-binding antibodies from the SLE donor and the healthy donor are shown in Table 2 and Fig. 4. A single antibody from an HIV-1-seropositive donor is included for comparison. Among the heavy chains of the SLE autoantibodies, five are from the V_H3 family and one from V_H1. Two are most closely related to the V_H26 germline gene, which encodes the 16/6 crossreactive idiotype and which has been associated with SLE autoantibodies (20), although a more fundamental role for V_H26 gene usage has been suggested (21). Two heavy chains (SI-22 and SI-40) are closely related to one another, having 5 nucleotide (4 amino acid) differences in the V_H gene and very similar HCDR3s. However, they appear to have arisen from different rearrangements, since SI-40 has an extra tyrosine in HCDR3. Furthermore, the first two amino acids of HCDR3, which are identical in the two Fabs, are

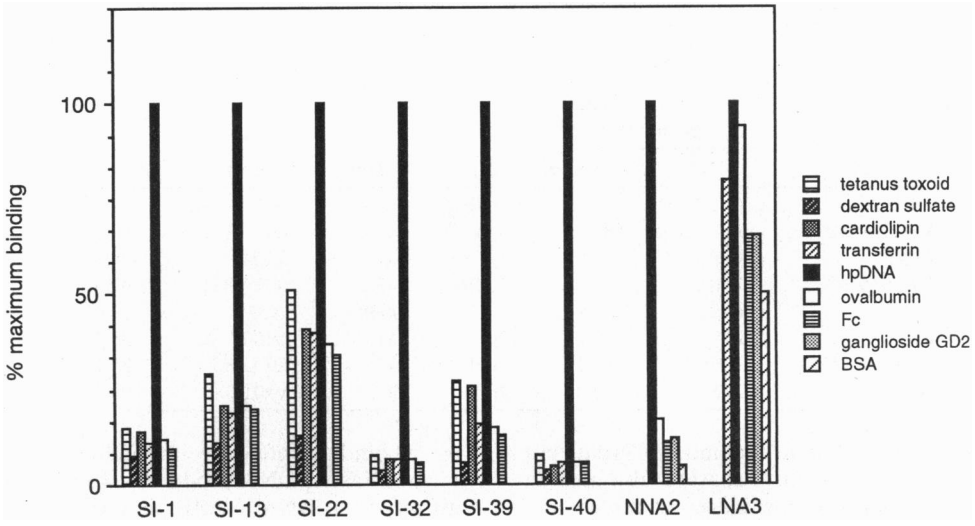

FIG. 2. Crossreactivity of Fabs to a panel of solid-phase antigens tested by ELISA. Fab concentrations were as follows: SI-1, 10 μg/ml; SI-13, 61 μg/ml; SI-22, 68 μg/ml; SI-32, 17 μg/ml; SI-39, 33 μg/ml; SI-40, 20 μg/ml; NNA2, 40 μg/ml; LNA3, 40 μg/ml. These concentrations were chosen to provide maximal absorbance values against DNA after 15 min. Values are reported as a percentage of the maximum absorbance attained. BSA, bovine serum albumin.

encoded by different codons. An alternative explanation is that one of the clones has arisen from the other through a crossover event *in vitro* (PCR crossover; refs. 22 and 23), but this is unlikely due to the pattern of differences between clones (in total there are 10 nucleotide differences and the use of different 5′ primers). The V_H sequence from the anti-DNA Fab from the healthy donor library is derived from the V_H6 family via extensive somatic hypermutation. A striking observation from Table 2 is the relatively high degree of homology of V_H and V_L genes from the SLE autoantibodies with their corresponding closest germline sequences. Using library methods, we have obtained the V_H genes of IgG1 antibodies against a wide selection of pathogens and generally observed homologies to the closest germline sequence in the range 85–95% [from 10 anti-HIV-1 gp120 antibodies, 87% (24); from 4 anti-cytomegalovirus antibodies, 89% (10); and from 10 anti-herpes simplex virus antibodies, 90.5% (25)]. Average homology for antibodies derived by cellular methods from 4 anti-HIV-1 gp120 antibodies was 89% (26) and from two anti-tetanus toxoid antibodies was 92.5% (27). The average homology for library-derived antibodies from 18 anti-thyroid peroxidase antibodies was 90% (28–30). In contrast, the average homology to germline of the V_H genes from the SLE library in Table 1 is 96.5%. Even more strikingly, the antibodies with the highest affinity for DNA and *Crithidia*-positive staining were the closest to germline (SI-1, -40 and -32 have homologies of 98%, 99%, and 98%, respectively). The anti-DNA Fab from the healthy donor had ≈92% V_H sequence homology with the V_H6 germline (Table 2).

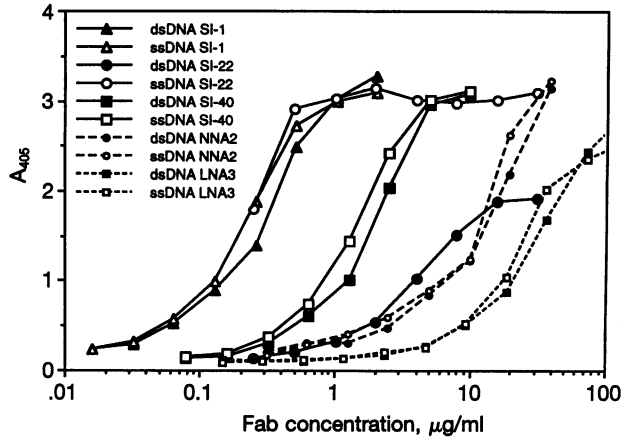

FIG. 3. Comparison of binding of purified Fabs from a SLE (SI), healthy (N), or HIV-1-seropositive (L) donor to solid-phase denatured DNA (single-stranded DNA, ssDNA) and dsDNA by ELISA.

The large number of studies on gene usage in anti-DNA autoantibodies have led to a consensus favoring an important role for somatic hypermutation in antibodies mediating DNA recognition and SLE pathology (e.g., refs. 18 and 31). DNA binding by unmutated or minimally mutated antibodies has also been described, however (32–34). One difficulty in making comparisons is that most studies provide little quantitative information on the strength of antibody binding to DNA. In our study, somatically hypermutated anti-DNA antibodies were identified, but they showed a trend toward lower affinities for dsDNA as assessed by several independent methods. To our knowledge only seven human anti-dsDNA IgG antibodies of demonstrated high affinity have been described previously (19, 35, 36). These anti-dsDNA antibodies, which were isolated from SLE patients by cellular methods, are the most appropriate comparison to our high-affinity anti-dsDNA Fabs. Winkler *et al.* (19, 35) described six high-affinity monoclonal anti-DNA IgG antibodies from SLE donors in which V_H homologies to germline varied between 94% and 99%. Van Es *et al.* (36) described a high-affinity anti-DNA antibody from a SLE donor which had 95% homology to its closest germline.

Basic residues have been suggested to be important for the interaction with DNA. Fig. 4 shows that a number of basic residues present in the V_H regions have apparently arisen by somatic mutation. However most of these are found in the Fabs with lower affinity for DNA (SI-13, SI-39, and NNA2). One of the most consistent features of Fig. 4 is the clustering of basic residues in HCDR3. This phenomenon has been described for many mouse anti-DNA hybridoma antibodies (reviewed in ref. 18) and the highest-affinity human anti-DNA hybridoma antibodies (35). The library antibodies with the highest affinity for DNA have either two (SI-40) or three (SI-1 and SI-32) arginines or lysines in HCDR3. There is a strong bias toward arginines (particularly) or lysines in the first four residues of HCDR3. While arginines are abundant in HCDR3 from murine DNA-binding antibodies, they are relatively rare in HCDR3 from other murine antibodies (37). Therefore, it may be that DNA binding arises whenever arginine is strongly represented in HCDR3. This is not the case for human antibodies, however; several anti-gp120 antibodies have two or

Table 2. Comparison of gene usage and structural homologies for heavy- and light-chain V regions (V_H and V_L) of anti-dsDNA Fabs from lupus (SI), healthy (N), and HIV-1-seropositive (L) donor libraries

	V_H gene usage					V_L gene usage				
			% homology with germline					% homology with germline		
Fab	V_H family	Nearest V_H	V_H DNA	V_H protein	J_H	V_L family	Nearest V_L	V_L DNA	V_L protein	J_L
SI-1	3	GL-16	98.1	96.7	J_H4b	κIII	kv325	97.6	95.1	$J_κ1$
SI-13	3	V_H26/V3-23	91.6	89.1	J_H4b	λ2	DPL11	96.1	91.8	$J_λ2$ or 3
SI-22	3	Cos3	97.8	94.6	J_H6b	λ1	lv1042	98.8	98.8	$J_λ2$ or 3
SI-32	3	V_H26/V3-23	97.8	97.8	J_H4b	λ3	IGLV3S1	99.6	98.6	$J_λ2$ or 3
SI-39	1	DP-75	94.9	89.1	J_H6b	κIII	kv325	97.6	95.1	$J_κ1$
SI-40	3	Cos3	98.9	96.7	J_H6b	λ1	lv1042	95.7	94.2	$J_λ2$ or 3
NNA2	6	V6-01/6-1G1	91.8	82.4	J_H5b	λ7	DPL18	95.1	91.0	$J_λ2$ or 3
LNA3	4	3d279d	93.8	92.2	J_H3b	κI	Vx012	90.4	84.5	$J_κ2$

more arginines in HCDR3 (24) but do not bind DNA (data not shown). The HCDR3s of the lupus Fabs are also rich in tyrosines, although there is no clear evidence of the characteristic tyrosine-rich motif arising from the Dxp'1 D gene segment, which is suggested to be important in DNA binding (reviewed in ref. 38).

The HCDR3s of SI-22 and SI-40, which bear a very close relationship to one another, also show a close relationship to a prototypic anti-dsDNA antibody from autoimmune MLR mice (3H9) (39), as shown in Fig. 4*B*. These similarities strongly suggest that HCDR3 motifs can be crucial in DNA binding. Further evidence for this viewpoint comes from studies on semisynthetic libraries (5) made up of antibodies in which most of the structure is retained but sections (specifically CDRs) are randomized by *in vitro* procedures. The studies described in ref. 5 used libraries based on a single tetanus toxoid-binding antibody with randomization of HCDR3. Panning against hpDNA produced two positive clones with high affinity for dsDNA. Electrophoretic mobility-shift assays carried out on one of the Fabs confirmed that it behaved as a specific DNA-binding protein. Hence, an antibody with high affinity and specificity for tetanus toxoid can be converted into antibodies with high affinity and specificity for DNA simply by manipulation of HCDR3. We therefore sought to establish whether DNA binding by the SLE antibodies could be mediated primarily through HCDR3 by subcloning the HCDR3s from these Fabs into the antibody used in the semisynthetic library studies, which has been shown permissive for DNA binding. Antibody grafted with HCDR3 from two of the highest-affinity SLE Fabs (SI-1 and SI-40) but not a third (SI-32) produced highly significant dsDNA binding as measured by ELISA (data not shown). Furthermore, the affinity

A

	FR1	CDR1	FR2	CDR2	FR3
VH26	LESGGGLVQPGGSLRLSCAASGFTFS	SYAMS	WVRQAPGKGLEWVS	AISGSGGSTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
SI-32	-----D----------	-----	----------	T----------	----------R
SI-13	--------K----------	K-N-N	----------	--DA--KT-N-------	----------K----------R
GL16	----------	-----	----------	S----------	----------
SI-1	----------G------	-----	----------	S----------	----------K----------I----R
Cos3	LVQSGGGVVQPGGSLRLSCAASGFTFS	SYGMH	WVRQAPGKGLEWVA	FIRYDGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
SI-40	LE----------	-----	----------	-------S---------	----------F-------V---------R
SI-22	-E----------T-------	-----	----------T	-------S--S------	----------F----------R
DP-75	VQSGAEVKKPGASVKVSCKASGYTFT	GYYMH	WVRQAPGQGLEWMG	WINPNSGGTNYAQKFQG	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR
SI-39	LE----------	-H-I-	----------	R----N---K-------	---I-----K--------S---E---------
V6-01	LEQSGPGLVKPSQTLSLTCAISGDSVS	SNSAAWN	WIRQSPSRGLEWLG	RTYYRSKWYNDYAVSVKS	RITINPDTSKNQFSLQLNSLTPDDTAVYYCAR
NNA2	----------T-------	R-N----	----------Q---	--H-T-R--H--EF---G	--S-TV----------L--D--------G
3d279d	LESGPGLVKPSETLSLTCTVSGGSIS	SGSYYWS	WIRQPAGKGLEWIG	RIYTSGSTNYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
LNA3	----------S-------	-------	----------	---S--------Y---	--AM-LG------F---T---PT---E-L---
TT	LEQSGAEVKKPGSSVKVSCRASGGTFN	NYAIS	WVRQAPGKGLEWMG	GIFPFRNTAKYAQHFQG	RVTITADESTGTAYMELSSLRSEDTAIYYCAR

B

	CDR3	FR4
SI-1	KRGRTTVSWGLVYFDY	WGQGTVVTVSS
SI-13	QKYRIDPGLDY	-----L-----
SI-40	ARWRDYYYYYMDV	--K--T-----
SI-22	ARWRD YYYYMDV	--K--T-----
3H9	ARSK YSYVMDY	
SI-32	GRGYSKSKYFDS	-----L-----
SI-39	GRRQLASRPYYYYLDV	--K--T-----
NNA2	ESDGRSSWFDP	-----L-----
LNA3	LEPGKYSYKPGPFDI	--R--M-----

C

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
lv1042	VLTQPPSVSGAPGQRVTISC	TGSSSNIGAGYDVH	WYQQLPGTAPKLLIY	GNSNRPS	GVPDRFSGSKSGTSASLAITGLQAEDEADYYC	QSYDSSLSG	
SI-22	E---E----------	----------	----------	------	----------	---------VV	FGGGTKLTVLG
SI-40	E---------P---------	----------A--	----------	--N----	----------TG-------	GTW-P---SW-	----------
DPL11	ALTQPASVSGSPGQSITISC	TGTSSDIGGYNYVS	WYQQHPGKAPKLMIY	EVSNRPS	GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	SSYTSSSTL	
SI-13	E- --P----------	--------A-----	---H--------I--	--FK---	----------	Q--D--LSGAV	----------
IGLV3S1	ELTQDPAVSVALGQTVRITC	QGDSLRSYYAS	WYQQKPGQAPVLVIY	GKNNRPS	GIPDRFSGSSSGNTASLTITGAQAEDEADYYC	NSRDSSGNH	
SI-32	-- ---V----------	---------P-	----------	-------	----------	---------WV	----------
DPL18	VVTQEPSLTVSPGGTVTLTC	ASSTGAVTSGYYPN	WFQQKPGQAPRALIY	STSNKHS	WTPARFSGSLLGGKAALTLSGVQPEDEAEYYC	LLYYGGA	
NNA2	EL----------	G---------S-A-	----------P---	A--S---	----------V---------D---	---QR-IKV	----------
kv325	VLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSP	
SI-1	E----------	---H---RA---	----------	-T-----	----------	----G--W	FGQGTKVELK
SI-39	E----------	---H---RA---	----------	-T-----	----------	----G--W	----------
TT	E----------	---H---RA---	----------	-T-----	----------	----G--W	----------
Vx012	QMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTP	
LNA3	EL----P-----------S-	-----VDA---	----N--T--Q---V	---T-H-	-----I----------H---------	-----PLLT	FGQGTKLDIK

FIG. 4. Amino acid sequences of anti-dsDNA Fab V_H and V_L domains from SLE (SI), healthy (N), and HIV-1-seropositive (L) donor libraries in comparison to closest known germline. All cationic residues are in boldface print, and those believed to have arisen from somatic mutation (deduced from comparison to closest germline sequence) are also underlined. The first two residues of framework region 1 (FR1) represent the restriction site incorporated by the upstream primer. The sequence of the tetanus toxoid (TT) antibody used in HCDR3 transplantation experiments is also shown. (*A*) V_H sequences. (*B*) Heavy-chain CDR3 and FR4 sequences. SI-22 and SI-40 are compared with murine 3H9 CDR3 sequence. (*C*) V_L sequences. The κ light chain in SI-1 and SI-39 corresponds to the insert (TT) present in the vector used in library construction.

constants of the grafted Fabs [designated TT(HCDR3, SI-1) and TT(HCDR3, SI-40)] for binding to the oligonucleotide probe in surface plasmon resonance studies were only slightly reduced compared with the parent SLE Fabs (Table 1). In addition, one of the grafted Fabs [TT(HCDR3, SI-1)] gave positive staining in the *Crithidia* assay. Thus the preeminence of HCDR3 in dictating DNA binding in at least two high-affinity SLE Fabs has been clearly demonstrated.

How meaningful is the study of library antibodies given that random recombination of heavy and light chains in library construction introduces uncertainty as to the correspondence of *in vivo* and cloned responses? In particular, the use of a vector-derived light chain by two of the Fabs from the SLE library calls into question the relevance of those Fabs. However, this unnatural pairing may not be of such importance, since the dominance of the heavy chain for DNA binding suggests that light-chain usage plays a lesser role in these recombinant Fabs. The relevance of the heavy chains is further supported by a correlation of idiotypes expressed on the recombinant anti-DNA Fabs and the corresponding serum anti-DNA antibodies (40). In addition, high-affinity *Crithidia*-positive antibodies were isolated from an SLE but not a healthy donor library. Thus, it seems likely that the cloned high-affinity antibodies are relevant to SLE.

In summary, we conclude that monoclonal antibody Fab fragments with moderate binding affinity to dsDNA can be isolated from libraries prepared from healthy and lupus donors. However, high-affinity Fab fragments were isolated only from the SLE library, and these antibodies showed minimal somatic hypermutation in the V genes. Basic residue-rich HCDR3s appeared to dominate DNA binding for two of three antibodies examined. Comparison of the behavior of the lupus antibodies and the corresponding HCDR3-grafted antibodies may allow dissection of the features responsible for DNA recognition and *in vivo* pathogenicity.

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