

The role of somatic mutation in determining the affinity of anti-DNA antibodies

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SUMMARY

Combinatorial antibody libraries were constructed from the spleen of a patient with concomitant systemic lupus erythematosus and idiopathic thrombocytopenia. Following selection of the libraries with DNA, a panel of 15 anti-DNA Fabs was isolated. Sequence analysis of these antibodies coupled with measurements of their affinities for ss- and dsDNA were used to investigate the role of somatic mutation in affinity maturation of the anti-DNA response. Examination of the germline genes used by these Fabs supports previous studies that suggest there is no restriction of the gene usage in the anti-DNA response. However, data are presented indicating that VH3 genes and the A27 V_κ paired with the J_κ1 may be over-expressed in the anti-DNA repertoire. Analysis of the role of somatic mutation in increasing affinity for DNA indicates that affinity maturation has occurred and suggests that the CDR1 and CDR2 of the heavy chain are of importance in this process.

Keywords human spleen lupus autoantibodies autoimmunity

INTRODUCTION

Systemic lupus erythematosus is characterized by high affinity antibodies to double-stranded DNA (dsDNA). The occurrence of such antibodies has been correlated with both disease flares and renal involvement [1–3]. However, the role of antigenic drive and the relative importance of somatic mutation in the production and pathogenicity of these high affinity antibodies are still unclear. Several studies have shown that human anti-DNA antibodies are somatically mutated, with a strong bias toward replacement mutations in the CDRs and increasing rates of mutation correlating with the switch from IgM to IgG [4–6]. This has been interpreted as evidence of affinity maturation [5,6]. However, increases in somatic mutation are not always apparent in such instances, e.g. Mannheimer-Lory and co-workers found no difference in the mutation rate of IgG and IgM anti-DNA antibodies [7]. The role of replacement mutations in increasing antibody affinity for DNA has also proved equivocal, with some studies indicating that mutations are important, whereas others find little correlation between affinity for dsDNA and mutation rate [8–11]. Certainly, it appears that some high-affinity anti-DNA antibodies can be encoded by genes that are essentially germ-line [8] and it is likely that the particular rearrangements of V, D and J segments in SLE patients determine the affinities of these antibodies [11].

The role of basic amino acids such as arginines and lysines in the CDR3s of such antibodies has been highlighted [8,9]. There are, however, other examples of anti-DNA antibodies where somatic mutations do appear to contribute to the affinity for dsDNA [10,11]. Thus, overall, it appears that there is evidence of somatic mutation, focused on the CDRs, in a proportion of anti-dsDNA antibodies from SLE patients. It is still not fully clear, however, if this is due to affinity maturation mediated by DNA or if the somatic mutations observed are incidental to affinity maturation in response to another antigen.

To resolve these issues, it is necessary to examine many individual anti-DNA antibodies and to try to correlate their affinity and specificity with germline gene usage and incidence of somatic mutation. The relative lack of human monoclonal anti-DNA antibodies (particularly of the IgG class) from SLE patients presents a problem here. Conventional techniques for generating human monoclonal antibodies tend to be inefficient and, in most cases, peripheral blood lymphocytes that contain relatively few IgG-producing B cells have been used [12]. An alternative is to generate human anti-DNA antibodies from SLE patients using repertoire cloning techniques, where DNA is used to select antibodies from a combinatorial library representing the heavy and light chain genes expressed by the patients' B cells. This method of sampling the human antibody response substantially increases the number of variable regions available for analysis [8,11].

This report seeks to add to the data on human IgG anti-DNA antibodies. We have used repertoire cloning techniques to

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construct a combinatorial library from the splenic lymphocytes of a patient with SLE and concomitant thrombocytopenia. By selecting against dsDNA, 15 IgG Fabs were isolated. We have analysed the sequences and affinities of these antibodies for ss- and dsDNA and in particular have examined the role of somatic mutation in increasing affinity for DNA.

MATERIALS AND METHODS

MRNA isolation and patient details

The spleen was taken from a 20-year-old-male with active SLE and concurrent idiopathic thrombocytopenia. Arthritis began at the age of five and he was found to be ANA positive. He was anti-Ro antibody positive at the age of 6. For the last 10 years frequent mouth ulcers were noted and for the last eight years he had had episodes of thrombocytopenia. He had been treated with oral prednisolone and danazol. The thrombocytopenia was usually steroid responsive although the platelet drop could be quite profound, reaching $9 \times 10^9/l$, for example. At the time of the splenectomy the platelet count was 322×10^9 per litre. His dsDNA antibody titre was 352 IU/ml. ANF was homogeneous at 1/320, and C3 and C4 levels were normal. He was also anti-Ro antibody positive but anti-La and anti-RNP antibody negative.

Total RNA was isolated from homogenized splenic tissue using an Ultraspec™ RNA isolation kit (Biotex Laboratories, Edmonton, Canada).

Library construction

Initial library construction used the pComB3HSS phage display vector (a gift from the Scripps Research Institute, La Jolla, USA) and was carried out as described previously [13–17]. After reverse transcription of 5 µg of RNA (SuperscriptII™, GIBCO, Paisley, UK) the γ 1 Fd region and κ light chains were amplified by PCR using C region and a previously described panel of V_H and V_κ first framework specific primers [17,18] and additional V_H and V_κ primers described in the Cold Spring Harbour Laboratory Course Manual, namely: V_H2f , (5'-CAGGTGCAGCTACTCGAGTCGGG-3'); V_H4f , (5'-CAGGTGCAGCTGCTCGAGTCGGG-3'); V_H6a , (5'-CAGGTACAGCTCGAGCAGTCAGG-3'); V_H6f , (5'-CAGGTACAGCTGCTCGAGTCAGGTCCA-3'); V_L1s , (5'-GACATCGAGCTCACCCAGTCTCCA-3'); V_L2a , (5'-GATATTGAGCTCACTCACTCAGTCTCCA-3'); V_L3b , (5'-GAAATTGAGCTCACG(G/A)CAGTCTCCA-3'). The heavy chain Fd and light chain PCR products were gel-purified, extracted (Wizard® PCR Preps DNA Purification System, Promega, Southampton, UK) and reamplified using extension primers with a 5'-poly (GA) tail to increase restriction enzyme digestion and cloning efficiency [16]. The light- and heavy chain PCR fragments were cut with *SacI*/*XbaI* and *SpeI*/*XhoI*, respectively (GibcoBRL, Paisley, UK) and ligated sequentially into pre-cut pComB3HSS vector [15,17]. After electroporation into *Escherichia coli*, XL-1Blue a light chain library of 3.3×10^7 independent clones was generated, followed by a combinatorial (Fd \times κ) library of 1×10^7 independent primary clones.

Library panning for dsDNA binding phage

The library was panned against biotinylated plasmid dsDNA [19]. Briefly, 8-well ELISA strips (Maxisorb, Nunc, Roskilde, Denmark) coated with streptavidin were incubated overnight at 4°C with biotinylated dsDNA (0.1 mg/ml), washed with distilled water and blocked with 3% (w/v) BSA in PBS. Successive rounds of panning

using freshly prepared phage were then performed as described [15] using increased washing stringency (1 \times , 3 \times , 6 \times , 10 \times) with TBS 0.05% Tween 20 to remove unbound phage at each stage.

Production of soluble Fabs

Soluble Fab fragments were generated using DNA isolated from a number of independent clones after the final round of panning, as previously described [15]. Bacterial lysates/supernates were then analysed for the presence of anti-dsDNA Fabs by ELISA. Additionally, two complementary oligomers coding for hexahistidine (His) tag (5'-CTAGCCACCATCATCACCACCATT-3'; 5'-CTAGAATGGTGGTGATGATGGTGG-3') were ligated into the COOH-terminal of the light-chain DNA, via the *XbaI* site, to allow purification of the fragments on a nickel agarose affinity column (Qiagen, Crawley, UK).

Enzyme-linked immunosorbent assay (ELISA)

Soluble Fabs were screened for dsDNA binding by ELISA. Microtitre wells were coated with dsDNA as described above, blocked with TBS containing 3% bovine serum albumin (BSA) for 1 h at 37°C and incubated with bacterial lysate/purified Fab for 1 h at 37°C. After three washes with TBS 0.05% Tween 20, wells were incubated at 37°C for 1 h with Fab-specific, HRP-labelled goat anti-human IgG (Sigma, Poole, UK) diluted 1 : 1000 in TBS-1% BSA. After washing as above, plates were developed with ABTS substrate tablets (Sigma) according to the manufacturer's instructions.

Determination of binding kinetics by surface plasmon resonance

Purified anti-dsDNA Fabs were analysed on the BIAcore system (BIAcore 2000, Biacore, Uppsala, Sweden). Biotinylated pure double-stranded plasmid DNA or single-stranded random oligonucleotide DNA was coupled to a streptavidin-coated sensor chip, yielding a change in response units (RU) of 1935. All determinations were performed at 25°C in 10 mM HEPES, 150 mM NaCl, 2 mM CaCl₂, 0.05% BIAcore surfactant P20, pH 7.4 (HBS-M), with a constant flow rate of 5 µl/min and an injected sample volume of 30 µl for all Fabs at a concentration of 10^{-7} M. The calculation of the off- and on-rates was performed with the BIAevaluation Program 2.0 (Pharmacia Biosensor, Uppsala, Sweden). Biacore was used to measure affinity rather than ELISA as this provides more detailed information to compare the relative affinities of the antibodies isolated.

Fab sequence determination

Nucleic acid sequencing of selected clones was carried out on a 373-A automated DNA sequencer (Applied Biosystems, Warrington, UK) using a *Taq* fluorescent dideoxy terminator cycle sequencing kit (Applied Biosystems), as described previously [17]. The V, J and D gene usage was determined using the VBASE program [20].

Statistics

Comparisons between group means were carried out using the student's *t*-test. Correlation was carried out by Pearson Correlation coefficients.

RESULTS

Variable region gene usage

After four rounds of panning, DNA from selected phage was sequenced and used to generate soluble Fab fragments. Fabs giv-

ing OD₄₀₅ readings of >0.5 against dsDNA as bacterial lysates/supernates in ELISA were selected for further analysis and purification. Table 1 shows the variable region genes used by 15 anti-DNA Fabs. Figures 1 and 2 show the deduced amino acid

sequences compared with those of the appropriate germline gene. Five of the nine V_H and two of the three V_L germline genes used have previously been described in association with anti-DNA antibodies [21]. J and D region germline genes have been

	* 20 * 40 * 60 *	
1.08	: QVQLVQSGAEVKKPGASVKVSKASGYTF	TSYDINWVRQATGQGLEWVGWMNPNSGNTGYAQKFGGRVTM : 70
P253	: E...LE.....T.....	F.....A.R.....C..... : 70
	80 * 100 *	
1.08	: TRNTSISTAYMELSSLRSED	TAVYYCAR----- : 98
P253	:	RARKGLDWGLGTLVTV : 115
	* 20 * 40 * 60 *	
1.46	: QVQLV-QSGAEVKKPGASVKVSKASGYTF	TSYYMHVWRQAPGQGLEWVGIIINPSGGSTSYAQKFGGRVT : 69
R5C08	: E...LEE..V.....S..N.....S.....V.....GA...N...L : 70	
R5C02	: E...L-E.....M.....GG.....R.....L.V.....R..... : 69	
R4C07	: E...L-E.....M.....GG.....R.....L.V..... : 69	
	80 * 100 * 120	
1.46	: MTRDTSTSTVYMELSSLRSED	TAVYYCAR----- : 98
R5C08	:	AGFQSSNRLFRTPWGHGTLVTV-- : 121
R5C02	:F.....Y..K....I...LTHGGAFD-----	IWGQTVVTV-- : 115
R4C07	:F.....Y..K....I...LISRGAFD-----	IWGQSTVVTVLF : 117
	* 20 * 40 * 60 *	
1-69	: QVQLVQSGAEVKKPGSSVKVSKASGGTF	SSYALSWVRQAPGQGLEWVGIIPIFGTANYAQKFGGRVTI : 70
R5C09	: E...LE.....A.....Y...IQND..N.....L...T...R..... : 70	
	80 * 100 *	
1-69	: TADESTSTAYMELSSLRSED	TAVYYCAR----- : 98
R5C09	: S..D.....V...HT.....VVTSVTTLDGFDVWGRGTMVT : 117	
	* 20 * 40 * 60 *	
3.07	: EVQLVE-SGGGLVQPGGSLRLS	CAASGFTFSSYWMSWVRQAPGKLEWVANI
R5C20	: ...L.E...V.R..R.....T.LS.H.....	KQDGSEKYYVDSVKGGRFT : 70
	80 * 100 * 120	
3.07	: ISRDNAKNSLYLQMN	SLRAEDTAVYYCAR----- : 98
R5C20	: ...SSN.A.....S.K...I...S.	DPEVGTPIITPDYRGQGILVTV : 121
	* 20 * 40 * 60 *	
3.09	: EVQLVE-SGGGLVQGRSLRLS	CAASGFTFDDYAMHWVRQAPGKLEWVSGISWNSGSIGYADSVKGRFT : 69
R4C12	:Q-..... : 69
R4C15	: ...L.E.....L.....DN.D..... : 70
R5C06	: ...L.E.....H.....V.....R.D.G..... : 70
R4C09	: ...L.E.....G..G.....V.....T.D...VS..G..M..... : 70
	80 * 100 * 120	
3.09	: ISRDNAKNSLYLQMN	SLRAEDTALYYCAKD----- : 99
R4C12	:	RSLEG---GFDPWQGALITVTV : 117
R4C15	:G.....	F.T.SILGK--DAFDIWQGTMTVTV-- : 118
R5C06	:N.....T...T...T.	GREVITLWVHPWQGTTLVTV-- : 120
R4C09	: L..E...V...D...PD...W...V.	GKSLWS--AFDDWQGTTLVTV-- : 118

Fig. 1. A comparison of the deduced VDJ region amino acid sequences of the heavy chains of 15 anti-DNA antibodies with those of the corresponding V_H germline genes. The CDRs are highlighted. The DNA sequences have been submitted to EMBL and are listed under the following accession numbers: R4C12 AJ306669, R4C15 AJ306670, R4C17 AJ306671, R4C03 AJ306672, R4C04 AJ306673, R4C07 AJ306674, R4C09 AJ306675, R5C20 AJ306676, R5C02 AJ306677, R5C06 AJ306678, R5C08 AJ306679, R5C09 AJ306680, R5C16 AJ306681, P212 AJ306681, P253 AJ306682.

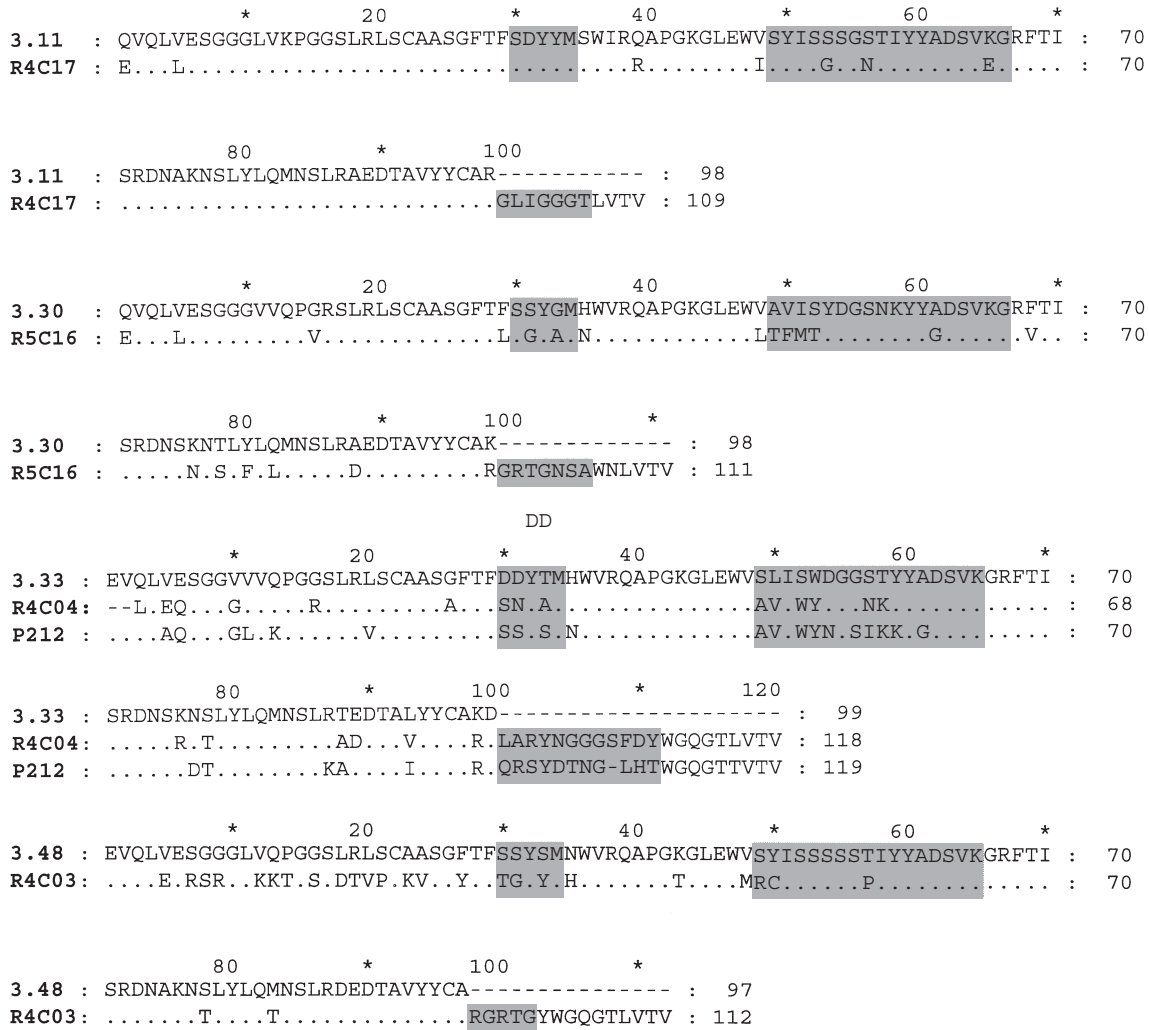


Fig. 1. Continued

assigned, although in many cases the CDR3 of the heavy chain appears to have arisen by a complex process and the accurate prediction of the germline genes used was not always possible.

Replacement to silent ratio and mutation rates

The mean replacement to silent ratio (R/S) for all the CDRs combined [Table 1], the CDRs 1 and 2 of the heavy chain combined [Table 2] and the CDRs 1, 2 and 3 of the light chain combined [Table 3] were greater than 3. The mean total mutation rate for the heavy chain was 7.58% (range 2.16–17.56; median 7.41) and the mean percentage of the heavy chain mutations that were transitions was 49.89% (range 28.1–77.8%; median 45.5%). The mean total mutation rate for the light chain was 8.68% (range 3.35–22.15; median 4.37) and the mean percentage of the light chain mutations that were transitions was 49.4% (range 27.3–81.8%; median 47.1%).

The R/S ratios for each CDR of the heavy chain for each antibody and their means are shown in Table 2 and for both CDR1 and CDR2 the mean was below 3. Table 4 shows the distribution of replacement to silent ratios for the heavy chains categorized according to the type of light chain used. Clear differences are seen between the groups, in that for antibodies that use A27 [1]

light chain there is no evidence of increased R/S ratios in either CDR1 and 2 of the heavy chain. This contrasts with antibodies that use A27 [2] light chain which show a marked focus of mutations in the CDR2 of the heavy chain compared with the CDR1 of the heavy chain. This pattern is also reflected in those antibodies that use B3, although the R/S ratio for the CDR2 of the heavy chain is not as high. In those antibodies that use A20 light chain, high R/S ratios are seen in both CDR 1 and 2 of the heavy chain. The R/S ratio for each CDR of the light chain for each antibody and their means are shown in Table 3 and for CDR1, 2 and 3 the mean was below 3.

The effect of replacement to silent ratio on antibody affinity

The K_a and K_d for the binding of all antibodies to ssDNA and dsDNA is shown in Table 5. Significant correlations were found between the combined R/S ratio for the CDR1 and 2 of the heavy chain and the K_a values for ssDNA and dsDNA (correlation coefficients = 0.8, $P = 0.0003$ and 0.86, $P = 0.0001$, respectively) and the K_d against ssDNA (correlation coefficient = -0.71 $P = 0.003$). By contrast, there was no significant correlation between the R/S ratio of the framework regions, when compared with K_a (correlation coefficients of -0.03 for ssDNA and 0.04 for

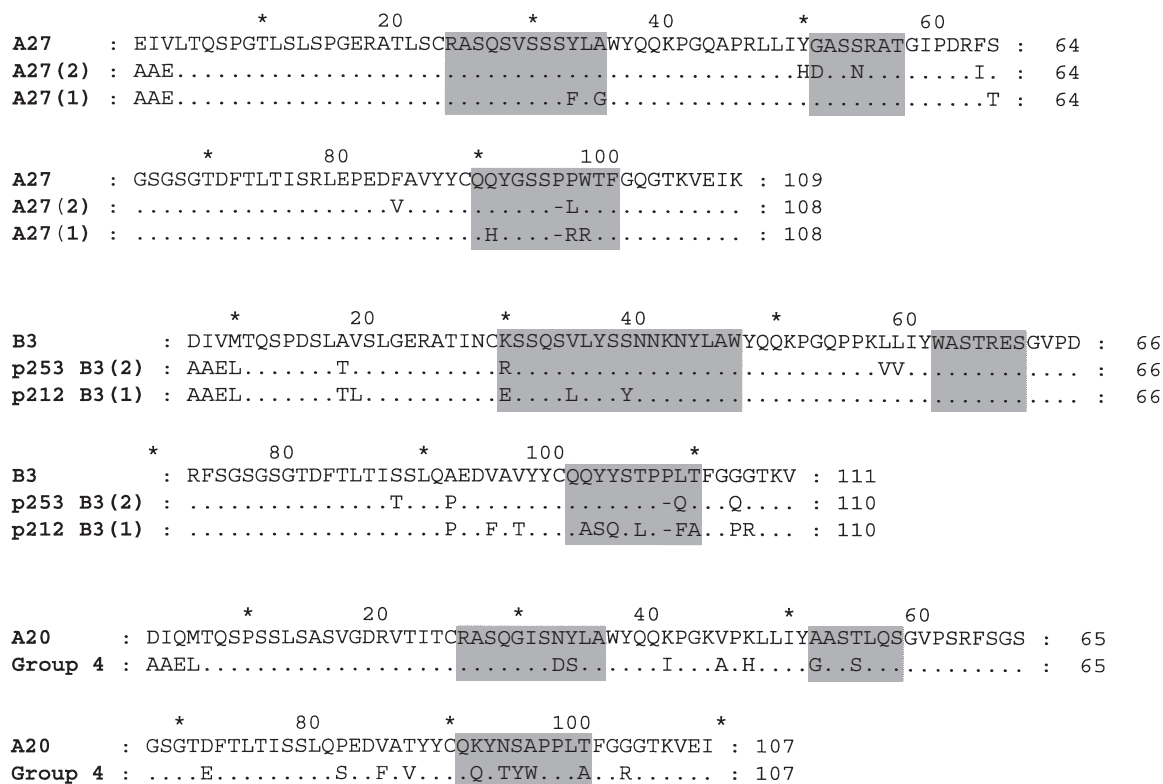


Fig. 2. A comparison of the deduced VJ region amino acid sequences of the light chains used by 15 anti-DNA antibodies with those of the corresponding $V_L V_J$ germline genes. The CDRs are highlighted. The DNA sequences have been submitted to EMBL and are listed under the following accession numbers: A27(1) AJ307664, A27(2) AJ307665, B3(1) AJ307666, B3(2) AJ307667, Group 4 AJ307668.

Table 1. Variable region genes used in 15 anti-DNA antibodies and the combined R/S ratio for all CDRs except CDR3 of the heavy chain. The variable region genes marked in italics have previously been described in IgG anti-DNA antibodies [12]. Variable region genes were assigned using VBASE [21] in the case of V_H , V_L , and J_L . D region genes and J_H genes were assigned by a combination of VBASE and manual comparison

Antibody code	V_H family	V_H Locus	D Locus	J_H Locus	V_L Locus*	J_L Locus	Total R/S ratio for the CDRs of the heavy and light chain combined
R5C08	1	<i>1-46</i>	D3-22	JH5B	A20	J κ 4	2-0
R4C17	3	<i>3-11</i>	D-5-24	JH4A	<i>A27(2)</i>	J κ 1	6-0
R4C09	3	3-9	D4-11/4/17	JH5B	<i>A27(1)</i>	J κ 1	3-5
R4C12	3	3-9	D6-?	JH5B	<i>A27(1)</i>	J κ 1	2-0
R5C02	1	<i>1-46</i>	D3-22	JH3B	<i>A27(1)</i>	J κ 1	1-4
P253	1	1-8	D1-1/D1-20	JH5B	<i>B3(2)</i>	J κ 4	7-0
R5C20	3	3-7			A20	J κ 4	2-7
R4C07	1	<i>1-46</i>	D3-22	JH3B	<i>A27(1)</i>	J κ 1	1-5
P212	3	3-33	D3-3	JH3B	<i>B3(1)</i>	J κ 4	4-3
R4C15	3	3-9	D7-27	JH3B	<i>A27(1)</i>	J κ 1	2-3
R4C03	3	3-48	D3-16	JH4B	A20	J κ 4	2-4
R5C06	3	3-9	D4-23	JH5B	<i>A27(2)</i>	J κ 1	4-3
R4C04	3	3-33	D3-3	JH4A	<i>A27(2)</i>	J κ 1	4-0
R5C16	3	<i>3-30</i>	D7-27	JH6a/b/c	A20	J κ 4	3-8
R5C09	1	<i>1-69</i>	D4-17	JH3A	A20	J κ 4	4-2
Mean							3-4

*Numbers in parenthesis indicate the light chain somatic variant used.

Table 2. The replacement to silent mutation ratios of the heavy chains for the 15 anti-DNA Fabs. Figures in italics are those for which there are no silent mutations

Antibody code	R/S ratio in CDR 1 and 2	R/S ratio in CDR 1	R/S ratio in CDR 2	R/S ratio in the framework region	Transition mutation rate
R5C08	1.3	1.0	1.5	2.5	42.9
R4C17	<i>3.0</i>	<i>0.0</i>	<i>3.0</i>	3	72.7
R4C09	<i>3.0</i>	0.0	<i>3.0</i>	0.7	35.7
R4C12	0.0	0.0	0.0	2.5	77.8
R5C02	1.0	<i>1.0</i>	0.7	4.5	66.7
P253	<i>3.0</i>	<i>2.0</i>	<i>1.0</i>	5	45.5
R5C20	2.5	2.5	0.0	2	38.5
R4C07	1.0	<i>1.0</i>	0.5	3.6	65.5
P212	<i>3.0</i>	<i>2.0</i>	<i>1.0</i>	3	47.8
R4C15	3.0	0.0	3.0	3	53.3
R4C03	3.0	4.0	2.0	3	37.3
R5C06	5.0	0.0	5.0	8	57.9
R4C04	7.0	<i>2.0</i>	5.0	2.75	33.3
R5C16	8.0	4.0	4.0	3.75	30.8
R5C09	9.0	6.0	3.0	1.625	28.1
Mean	3.4	1.7*	2.2	3.2	48.9

*Probability value, $P > 0.003$ when compared with combined R/S ratio for CDR1 and 2; not significant when compared with the R/S ratio for CDR2.

Table 3. The replacement to silent mutation ratios and homologies with germline genes of the light chains for the 15 anti-DNA Fabs. Figures in italics are those for which there are no silent mutations

V _L	J _κ	Combined R/S ratio for CDRs 1, 2 and 3	R/S ratio for CDR1	R/S ratio CRD2	R/S ratio CRD3	R/S ratio in the framework region	Transition mutation rate
A27(1) ¹	J _{κ1}	2	1	0	3	0.5	27.3
A27(2) ²	J _{κ1}	3	0	2	<i>1</i>	2	81.8
A20	J _{κ4}	2.75	3	2	2	1.7	50.0
B3(1) ³	J _{κ4}	3.5	3	0	3.67	3.8	41.0
B3(2) ⁴	J _{κ4}	4	2	0	2	4.0	47.1
Mean		3.05	1.8	0.8*	2.33	2.4	49.4

*Probability value, $P > 0.005$ when compared with total ratio of the CDRs and > 0.03 when compared with the CDR3, but not significant when compared with the CDR1. ¹Somatic variant of A27/J_{κ1} light chain used by antibodies R4C07, R4C09, R4C12, R4C15 and R5C02. ²Somatic variant of A27/J_{κ1} light chain used by antibodies R4C04, R4C17 and R5C06. ³Somatic variant of B3/J_{κ4} light chain used by antibody P212. ⁴Somatic variant of B3/J_{κ4} light chain used by antibody P253. The sequence differences between these light chains are described in Fig. 2.

dsDNA) or K_d (correlation coefficients of -0.18 for ssDNA and 0.18 for dsDNA). This strong correlation between affinity and somatic mutations is due to increased numbers of replacement mutations rather than decreasing numbers of silent mutations in the combined CDR1 and 2 of the heavy chain (correlation coefficients of 0.63 $P = 0.01$ for ssDNA and 0.74 $P = 0.002$ for dsDNA when compared for k_a , and -0.73 $P = 0.002$ for ssDNA when compared for kDa) (data not shown). No such correlation was seen when the light chain was studied.

DISCUSSION

In this study, we have used repertoire cloning to select 15 anti-DNA IgG antibodies from the splenic lymphocytes of a patient with SLE. It is well documented that these techniques can be used to isolate high affinity antibodies with the same antigen binding

specificity as the donor serum [16,22]. Nevertheless, a persistent criticism of these methods is that, as the heavy and light chain genes are randomly combined, the pairings observed in the antibodies selected are not necessarily those that occur *in vivo*. Whilst this is acknowledged, it should be noted that chain-shuffling experiments on antibodies isolated from libraries indicate that the heavy and light chain pairings required to generate anti-dsDNA specificities are relatively non-promiscuous [11,23]. The technique provides an efficient means of generating human antibodies and, coupled with an analysis of their binding properties, provides valuable information on the elements of the antibody response that may be used in the expression of IgG anti-DNA antibodies.

Analyses of variable gene use have, in this and other studies [21], failed to find any preference in the germline genes used. However there is a preponderance of V_H3 genes in the IgG anti-DNA Fabs described here and by Rahman *et al.* [21] (64% of 42

Table 4. A comparison of the mean replacement to silent ratios of the heavy chains paired with specific light chains. Antibodies using the different somatic variants of the A27/J κ 1 light chain are treated as two separate groups. As only two antibodies used the somatic variants of the B3/J κ 4 light chain, these they are treated together. Mean \pm s.e.m.

	Number of antibodies	R/S ratio for heavy chain CDRs 1 and 2	R/S ratio for CDR1	R/S ratio for CDR2
A27 (1)	6	2.83 \pm 0.27	1.33 \pm 0.19	1.69 \pm 0.12
A27 (2)	3	5 \pm 0.33	0.67 \pm 0.19	4.33 \pm 0.19
B3	2	2.97 \pm 0*	0.53 \pm 0*	2.88 \pm 0*
A20	4	4.55 \pm 0.37	3.50 \pm 0.19	2.10 \pm 0.15

*The numbers of mutations in both heavy chains was identical. For an explanation of A27 (1) and A27 (2) see the legend to Table 3.

antibodies and Fabs), compared with current estimates for all used antibody genes (approximately 40% of 1200) [24]. The number of human IgG anti-DNA antibodies that have been analysed, however, is still relatively small and clearly the nature of the immunoglobulin repertoire in SLE patients and its difference from normal individuals requires further study.

From the studies summarized in [21] and those reported here, a total of eight light chains that use the V κ gene A27 have now been described in IgG anti-DNA antibodies/Fabs. Of the seven in which the J region can be attributed, six are paired with J κ 1. Studies on the antibody repertoires of normal and SLE patients indicate that J κ 1 occurs in 20–30% of all antibodies [25,26]. This would suggest a preferential pairing of A27 and J κ 1 in anti-DNA antibodies, an observation that requires further investigation.

Although it has been suggested that antigen driven somatic mutation is important in the development of human anti-DNA antibodies, very few studies have been able to correlate the accumulation of mutations with changes in affinity (summarized in [5,6]). Studies that use site-directed mutagenesis to revert anti-DNA antibodies to their germline equivalents show contradictory data with regard to a role for somatic mutation in determining affinity [9,10]. Here, the replacement to silent ratios of greater than 3 observed in the combined CDRs of 10 of the 15 heavy chains and three of the light chains strongly indicate that an antigen driven process is occurring. This is further supported by the mean purine to pyrimidine transition rate of 48.9% for the heavy chain and 49.4% for the light chain even though somatic hypermutation is inherently biased in favour of purine to pyrimidine transitions, with a rate of 66% expected by random [27]. Taken together, these findings imply that affinity maturation in response to antigen has occurred in the development of these antibodies. It has been shown that, in antibodies generally, the CDR1 of the heavy chain has a higher inherent replacement to silent ratio than CDR2 [28]. Here, however, the mean replacement to silent mutation ratio in the CDR1 of eight of the 15 H chains is lower than in CDR2 [Table 2]. It is possible that antigen selection determines the lower replacement to silent ratio in the CDR1 of the heavy chain, although further studies are needed.

Analysis of affinity measurements shows a strong correlation between the R/S ratio in the CDRs 1 and 2 of the heavy chain and

Table 5. The affinity constants K_a and K_d rates for 15 anti-DNA antibodies as determined by BIAcore analysis

Antibody code	ss $K_a \times 10^8$	ss $K_d \times 10^{-7}$	ds $K_a \times 10^8$	ds $K_d \times 10^{-7}$
R5C08	0.30	0.34	0.05	2.16
R4C17	0.56	0.18	0.08	1.18
R4C09	0.16	0.64	0.17	0.60
R4C12	0.10	1.04	0.19	0.54
R5C02	0.16	0.62	0.19	0.53
P253	0.19	0.52	0.23	0.44
R5C20	0.15	0.68	0.23	0.43
R4C07	0.19	0.52	0.26	0.39
P212	0.18	0.56	0.27	0.38
R4C15	0.19	0.53	0.27	0.37
R4C03	0.25	0.39	0.41	0.24
R5C06	0.34	0.29	0.42	0.24
R4C04	0.28	0.36	0.48	0.21
R5C16	0.58	0.17	0.62	0.16
R5C09	0.81	0.12	0.83	0.12

K_a for both ss- and dsDNA and for K_d for ssDNA. This contrasts with a lack of correlation when the R/S ratio of the heavy chain framework regions are compared with both K_a and K_d . Whilst it is unlikely that all of these mutated residues are involved in DNA binding, the increasing level of mutation in the heavy chain CDRs with increasing affinity of the antibodies is quite striking. However, as only two of the antibodies (R5C20 and R4C07) appear to be clonally related, it is difficult in this study to determine the contribution of the CDR3 of the heavy chain, or of individual amino acids to affinity. A difference between the related antibodies is the presence of an arginine in place of a glycine in the CDR3 of the heavy chain of the slightly higher affinity R4C07 [Fig. 1]. Arginine and lysine have been reported to be important in determining the affinity of anti-DNA antibodies [8,9]. In general, however, whilst this panel of antibodies contains several basic amino acids there appears to be no direct relationship between affinity and the number of basic amino acids. Clearly, future site-directed mutagenesis studies will be important in determining the role of individual amino acids in DNA specificity.

When heavy chains are classified according to the light chain used, then it appears that somatic mutation may be more important in some groups than others. Notably there is a much higher R/S ratio in the heavy chain of antibodies that use the A27 [2] light chain when compared with those that use A27 [1], suggesting that the light chain plays a more prominent role in the latter group. This pattern suggests that mutations in each CDR can play a significant role in determining the specificity and affinity of anti-DNA antibodies, depending on their context within the structure of the combining site. Modelling of the Fab:DNA interaction and 3-dimensional structural studies on the Fabs would obviously provide further information here.

In conclusion the germline genes used by this panel of antibodies show characteristics consistent with other human anti-DNA antibodies and studies on variable gene usage in patients with SLE. Evidence is also provided that the high replacement to silent ratio observed here and by others is significantly correlated with increasing affinity. However, the role of individual mutations in determining affinity for DNA must be verified, e.g. by struc-

tural studies or mutagenesis. The correlation between mutation and increasing K_a for dsDNA indicates that affinity maturation of the response to dsDNA has occurred. This panel of antibodies provides an excellent resource to investigate antigen: antibody interactions in anti-DNA antibodies. The libraries will also provide a resource to study the antibody response to other autoantigens, such as platelets; these studies are currently in progress.

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