Immunology 1993 **78** 371–378

Primary sequence and location of the idiotopes of V-88, a DNA-binding monoclonal autoantibody, determined by idiotope scanning with synthetic peptides on pins

N. A. STAINES, F. J. WARD, A. N. DENBURY, J. MITCHINER, O. HARTLEY, D. EILAT,* D. A. ISENBERG† & S. BANSAL‡ *Infection and Immunity Research Group and ‡School of Pharmacy, King's College London, †Bloomsbury Rheumatology Research Unit, University College London, U.K. and *Hadassah University Hospital, Jerusalem, Israel*

Accepted for publication 11 October 1992

SUMMARY

In this study, the primary sequence and location of the idiotopes of monoclonal antibody (mAb) V-88 have been examined. V-88 was derived from an adult (NZB × NZW)F_1 mouse, has been partially defined previously with polyclonal anti-idiotype antisera, and is a member of the 16/6 idiotype (Id) family. From the inferred primary amino acid sequence of the antibody, sets of hexapeptides, overlapping by five residues, were synthesized on pins and used to scan the expression of epitopes (idiotopes) in the V regions of the light and heavy chains. A heterologous rabbit antiserum raised against the native antibody V-88, and absorbed to make it idiotype specific, was found to react with eight major epitopes distributed between the V_H and V_L regions. Half of these determinants mapped to the complementarity determining regions, with the others in framework sequences. Thus, the idiotype of antibody V-88 comprises, at least in part, continuous linear idiotopes in both hypervariable and framework areas. The process of absorbing the anti-idiotype antiserum on normal mouse immunoglobulin removed much of the background antibody activity against V region peptides, but left the activity against the dominant idiotopes. The sequence of a major idiotope, VATISG, in the FW2/CDR2 V_H region is homologous to sequences of human antibodies that express the 16/6 idiotype, suggesting that Id.16/6 is at least in part defined by this region of the antibody. The same V_H area is also homologous to sequences in bacterial and mammalian heat-shock proteins (hsp60–65). Thus there may be a functional link through idiotype connections, especially those involving Id.16/6, between anti-bacterial responses and production of autoantibodies, and some bacterial antigens may function indirectly as superantigens for B cells.

INTRODUCTION

The idiotype (Id) of an antibody is defined by anti-Id antibodies that react with epitopes, known as idiotopes, in its variable (V) region. The precise relationship of the idiotype to the specificity of an antibody is often unknown, but it is clear that some idiotopes are an integral part of the antibody paratope because anti-Id antibodies and antigen compete with each other for antibody binding. Other idiotopes are outside the paratope because such competition does not occur in all systems. From reactions of anti-Id antibodies with denatured heavy (H) or light (L) chains it is evident that some idiotopes are defined by continuous peptide sequences. However, not all can be recognized in this way, so other idiotopes are defined by discontinuous structures.

Some Id, especially those associated with responses to small haptens appear to have simple patterns of genetic inheritance. This indicates that primary nucleotide, and hence peptide, sequences can define an Id, although crystallographic studies show some idiotopes to be discontinuous structures.[1] A common approach to define the structure of Id involves comparison of primary sequences of antibodies that share Id, and in some cases small peptide idiotope motifs have been described (e.g. ref. 2).

In systemic lupus erythematosus (SLE) the production of autoantibodies against a range of cellular antigens characterizes the disease in both humans and mice. In particular, those

Abbreviations: CFA, complete Freund's adjuvant; DNA, deoxyribonucleic acid; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; FW, framework; HRP, horseradish peroxidase; hsp, heat-shock protein; Id, idiotype; IFA, incomplete Freund's adjuvant; Ig, immunoglobulin; mAb, monoclonal antibody; PBS, phosphate-buffered saline; RNA, ribonucleic acid; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA.

Correspondence: Dr N. A. Staines, Infection and Immunity Research Group, King's College London, Campden Hill Road, London W8 7AH, U.K.

autoantibodies that react with DNA have been intensively studied, and in SLE they appear to contribute to pathology through their involvement in forming immune complexes that associate with basement membranes in the kidney, brain and skin (reviewed in ref. 3). They have a diverse range of fine specificities for different epitopes on DNA.[4-6] Their Id have been defined by polyclonal heteroantisera or monoclonal antibodies (mAb). In general, the Id are not limited to a single antibody, and are therefore described as public, common or cross-reactive in their expression (reviewed in ref. 7). In many cases, the anti-Id antibodies inhibit the binding of the antibody to DNA[7] although this does not prove that the Id is associated solely with the paratope.

Monoclonal antibody V-88 is one of a collection derived from an adult mouse with SLE.[6] The antibody itself binds preferentially to single-stranded (ss) DNA, but also reacts with double-stranded (ds) DNA, some forms of RNA and cytoskeletal and nuclear structures.[6,8] Its Id has been defined by anti-Id antisera made both in guinea-pigs and rabbits and is in part related to the antibody paratope because anti-Id antibodies block its binding to DNA.[9] The mAb expresses the idiotype of mAb 16/6 (referred to as Id.16/6), originally defined upon a human DNA-binding autoantibody, making V-88 one of a family of Id.16/6$^+$ antibodies.[10] The Id.V-88 itself has been found on other mouse DNA-binding mAb,[9] immunoglobulins in the sera of human patients with a variety of autoimmune and infectious diseases and antibodies detected in the salivary glands of patients with Sjogren's syndrome.[11] Studies of SLE[9] and accelerated lupus disease in mice[12] have indicated that Id.V-88 is especially associated with the pathological process. It is present, for example, on immunoglobulins in renal biopsies from one-third of lupus patients.[11]

In order to define the primary structure of the idiotypes of the mAb V-88 we have prepared overlapping synthetic peptides, immobilized on pins, and have used these in an epitope scanning system.[13] This technology has been applied before to the analysis of epitopes in many other protein antigens, but has not, to our knowledge, been used before to map antibody idiotopes.

MATERIALS AND METHODS

mAb V-88

The derivation and properties of the DNA-binding mAb V-88 have been described previously;[6,8,9,12] it is an IgG1κ antibody from an adult female (NZB × NZW)F_1 mouse.

Sequence analysis of antibody variable regions

Purification of hybridoma RNA, end-labelling of oligonucleotide primers with [γ-^{32}P]ATP, synthesis of first strand cDNA, and chemical sequencing were carried out as described by Eilat *et al.*,[14] based on the methods of Caton *et al.*[15]

Anti-idiotype reagents

Rabbits were immunized with purified mAb incorporated in complete Freund's adjuvant (CFA) injected intradermally.[9,12] Repeat immunization was in incomplete Freund's adjuvant (IFA). When a high titre of antibody was found against the immunizing mAb, terminal sera were collected and absorbed by repeated passage over a column of normal mouse immunoglobulin (Ig) attached, according to the manufacturers' instructions, to CNBr Sepharose 4B (Pharmacia, Uxbridge, U.K.). This process was continued until no further activity against normal mouse Ig could be removed.

Serological procedures

Antibody activity against ssDNA and dsDNA was assayed by an enzyme-linked immunosorbent assay (ELISA).[6]

The anti-Id and anti-Ig activities of the rabbit antisera were titrated in an ELISA,[9] in which purified mAb (Id$^+$) or normal mouse IgG was immobilized in wells of microtitre plates (Nunc, Roskilde, Denmark). Binding of rabbit antibodies was quantitated with a peroxidase-labelled goat anti-rabbit IgG reagent (Sigma, Poole, U.K.).

Synthesis of immobilized peptides on pins

This followed procedures described by Geysen and colleagues[13] using an Epitope Scanning Kit (Cambridge Research Biochemicals, Northwich, U.K.). In all cases, Fmoc derivatives were used and oligopeptides (each with an acetylated amino terminus) were synthesized on polyethylene pins from their anchored carboxy termini. Sets of hexapeptides, overlapping by five residues, were prepared in duplicate corresponding to the V_H and V_L sequences of mAb V-88.

Substitution peptides and truncated peptides of selected regions were also synthesized as described later in the text. Software programs were used according to the manufacturers' instruction.

Epitope scanning

Blocks of pins with immobilized peptides were used in ELISA according to the manufacturers' instructions. The dilutions (in volumes of 175 μl/well) of test sera used and the times for which pins were incubated in them are indicated in the figure legends. The read out employed conventional species-specific peroxidase-linked anti-Ig reagents, and the final colour product was generated from oxidation of azino-di-3-ethyl-benzthiazolin-sulphonate (ABTS) and absorbance results given as A_{405nm} values.

RESULTS

Primary sequence of mAb V-88

The nucleotide and inferred amino acid sequences of mAb V-88 are shown in Fig. 1. The heavy chain sequence identifies this as a member of the V_H 7183 family,[16] closely resembling the V_H 37.1 and V_H 283 germline genes of the BALB/c mouse.[17] The few nucleotide differences between these germline genes and the V-88 sequence could be due to a closely related germline gene, to allelic differences between BALB/c and (NZB × NZW)F_1 mice or to somatic mutations. Several other anti-DNA heavy chains encoded by genes of the 7183 family of the mouse have been documented.[18,19]

The light chain sequence of the V-88 mAb belongs to the VK1A subgroup[20] and is probably encoded by the prototype K5.1 germline gene.[21] The eight nucleotide differences observed between this germline gene and the V-88 sequence probably result from somatic point mutations. Although the (NZB × NZW)F_1 allele of this germline gene has not been isolated, a comparison with another (NZB × NZW)F_1 VK1A sequence, PME77,[22] suggests that the nuleotide changes do not arise from allelic differences between the two strains of mice.

88 VH Sequence

```
                                                                GAA GTG ATG
                                                                 E   V   M

TTG GTG GAG TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT
 L   V   E   S   G   G   G   L   V   K   P   G   G   S   L   K   L   S   G   A   A   S

GGA TTC ACT TTC AGT AGC TAT GTC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG GAG TGG
 G   F   T   F   S   S   Y   V   M   S   W   V   R   Q   T   P   E   K   R   L   E   W

GTC GCA ACC ATT AGT GGT GAT GGT GGT AGT TAC ACC TAC TAT CCA GAC AGT GTG AAG GGT CGA TTC
 V   A   T   I   S   G   D   G   G   S   Y   T   Y   Y   P   D   S   V   K   G   R   F

ACC ATC TCC AGA GAC AAT GCC AAG AGC ACC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC
 T   I   S   R   D   N   A   K   S   T   L   Y   L   Q   M   S   S   L   R   S   E   D

ACG GCC TTG TAT TAC TGT GCA AGA CTT CCC TAC TAT AGT AAC TAC GCC TGG TTT GCT TAC TGG GGC
 T   A   L   Y   Y   C   A   R   L   P   Y   Y   S   N   Y   A   W   F   A   Y   W   G

CAA GGG ACT CTG GTC ACT
 Q   G   T   L   V   T
```

88 VL Sequence

```
GAT GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAC CAA GCC TCC ATC TCT
 D   V   V   M   T   Q   T   P   L   S   L   P   V   S   L   G   D   Q   A   S   I   S

TGC AGA TCT AGT CAG AGC CTC GAA CAC AGT AAT GGA TAC ACC TAT TTA CAT TGG TAC CTG CAG AAG
 C   R   S   S   Q   S   L   E   H   S   N   G   Y   T   Y   L   H   W   Y   L   Q   K

CCA GGC CAG TCT CCA GAA CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT GGG GTC CCA GAC AGG
 P   G   Q   S   P   E   L   L   I   Y   K   V   S   N   R   F   S   G   V   P   D   R

TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC AGA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG
 F   S   G   S   G   S   G   T   D   F   R   L   K   I   S   R   V   E   A   E   D   L

GGA ATT TAT TTC TGC TCT CAA AGT ACA CAT GTT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA
 G   I   Y   F   C   S   Q   S   T   H   V   P   Y   T   F   G   G   G   T   K   L   E

ATA AAA CGG GCT
 I   K   R   A
```

Figure 1. The gene sequence and inferred amino acid sequence of DNA-binding mAb V-88 derived from a (NZB × NZW)F_1 mouse. The CDR sequences are underlined and correspond to residues 31–35 (CDR1), 50–65 (CDR2) and 95–102 (CDR3) in V_H 88, and 24–34 (CDR1), 50–56 (CDR2) and 89–97 in V_L 88.

The VK1A gene has previously been found to encode several light chains of anti-DNA autoantibodies derived from (NZB × NZW)F_1 and MRL mice;[18,22,23] however the combination of V_H 7183 and VK1A light chain in a single anti-DNA antibody has not been documented previously.

The rabbit anti-Id.V-88 antiserum recognizes V region epitopes defined by linear hexapeptides

The rabbit anti-V-88 antiserum identified several epitopes when reacted with the V_H (Fig. 2A) and V_L (Fig. 3A) hexapeptides. The background reactivity was relatively high, but it was obvious that several areas in both CDR and framework (FW) regions defined epitopes for the rabbit antibodies.

After the anti-V-88 antiserum had been exhaustively absorbed upon normal mouse Ig, it reacted selectively with the mAb in ELISA. In the epitope scanning system its background reactivity was reduced and it reacted sharply with several epitopes in both chains.

In the H chain (Fig. 2A), the dominant epitopes were found to be in HCDR1, defined by the sequence SSYVMS (single letter amino acid code), and in HFW2/HCDR2, defined by a sequence EWVATISG. HFW3 contained several peptides (from number 49 to 67) that gave strong signals. In the L chain (Fig. 3A), the dominant epitope was defined by FSGSGSG in LFW3. Epitopes were also found in LFW1 (ASISCR), LCDR1 (SSQSLE and EHSNGY) and LCDR3 (QSTHVP and THVPYT---). Most of these, especially the reactive hexapeptides in V_H, were relatively hydrophobic according to the Hopp and Woods formula.[24]

Comparing the reactions of the unabsorbed with the absorbed serum, it was obvious that the former did react with a few epitopes not identified by the latter. The process of absorption itself removed a significant amount of antibody activity against epitopes in CDR and FW of both V_H and V_L regions. The serum was unreactive with LCDR2 in the assay conditions used.

In other experiments (not shown), it was confirmed that the peroxidase-linked goat anti-rabbit Ig developing reagent used in the epitope scanning ELISA did not react significantly with any of the V_H or V_L peptides ($A_{405nm} \leqslant 0{\cdot}150$).

Normal sera lack significant amounts of anti-V region peptide antibodies

A pool of sera from young normal rabbits gave a general background reactivity with V_H (Fig. 2C) and V_L (Fig. 3C) peptides. Even when the test incubation was extended, there were few reactions that were significantly above background, and none corresponded with those recognized by the anti-V-88 antiserum. As a control this indicates that the antibodies in the anti-V-88 antiserum were produced as a result of immunization.

Analysis of the primary structure of the dominant H-chain epitope

Analysis of the VATISG sequence with a set of substitution peptides (Fig. 4A) showed that the amino terminal Val or Ala

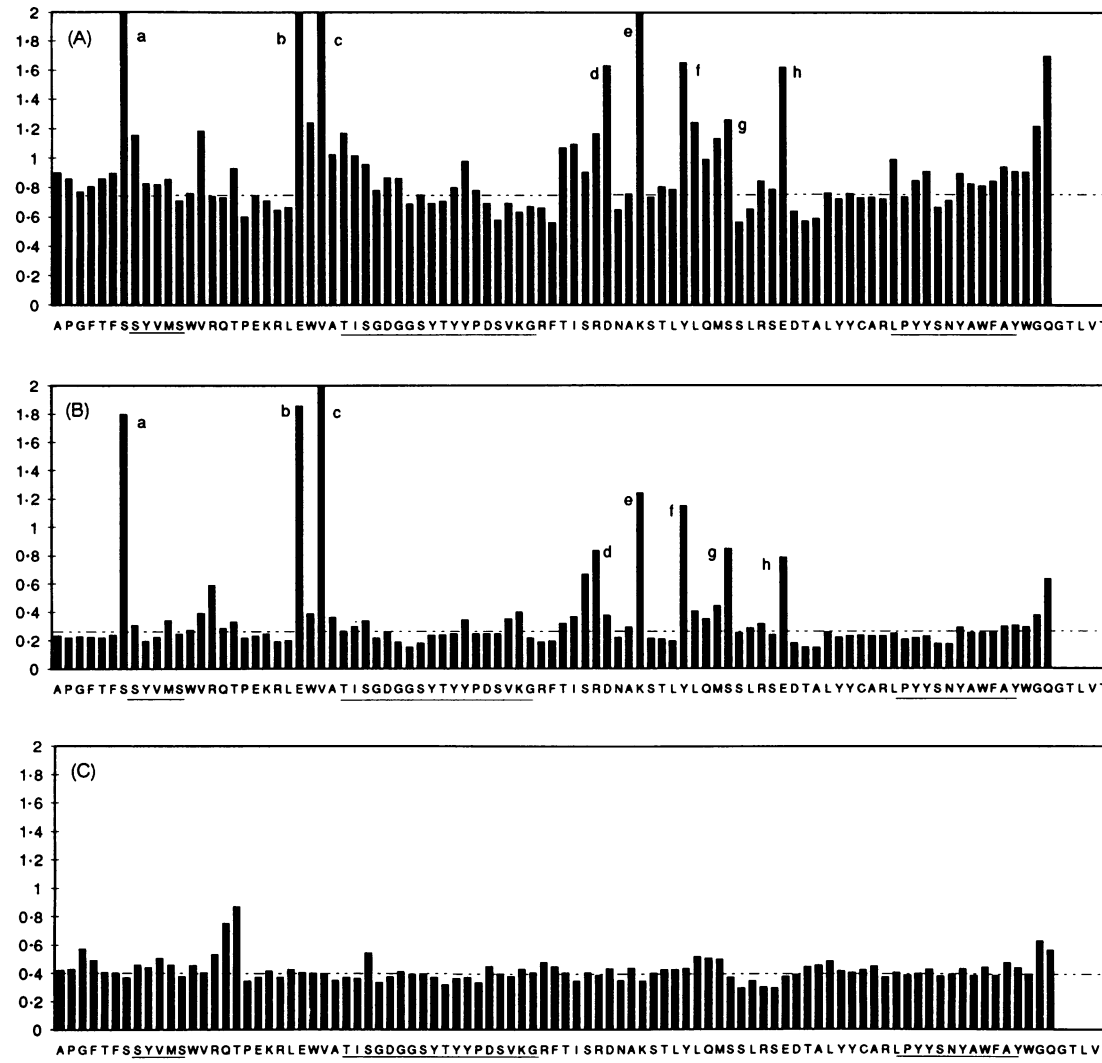

Figure 2. The idiotopes of the heavy chain of mAb V-88 identified by the rabbit anti-idiotype antiserum. Synthetic hexapeptides, overlapping adjacent peptides by five residues, were synthesized on pins, and reacted in ELISA with a rabbit anti-V-88 antiserum before (A) and after (B) absorption with normal mouse immunoglobulin. The unabsorbed serum was diluted 1/1000, reacted ~ 17 hr at 4° with the pin peptides; the secondary reagent was horseradish peroxidase (HRP)-labelled anti-rabbit IgG (Jackson Labs, Bar Harbor, ME) diluted 1/2000 and reacted for 1 hr at 37°. The final enzymic colour reaction (ABTS) was allowed to proceed for 20 min at room temperature. The absorbed anti-V-88 antiserum was diluted 1/1000 relative to the original serum. As control, a pool of sera from young unimmunized rabbits is shown (C); this was diluted 1/1000 and the reactions conducted under the same conditions. The amino acids at the amino termini of each peptide are indicated, and the final five beyond the V_H carboxy terminus are also shown. The CDR are identified by underlining of residues. Control background binding is indicated by the broken horizontal line and corresponds to the mean +2 SD of the lowest 25% of all values in each scan. At the time of the experiments, the amino terminal part of FW1 had not been sequenced (cf. Fig. 1); thus this is not represented in the hexapeptide arrays. The major lettered idiotopes are: (a) SSYVMS, (b) EWVATI, (c) VATISG, (d) RDNAKS, (e) KSTLYL, (f) YLQMSS, (g) SSLRSE, (h) EDTALY.

residues or the carboxy terminal Ser or Gly residues could be replaced with little effect. On the other hand, if Thr was replaced with either Ala, Asp or Gly, no binding was seen. Likewise replacement of Ile with other residues either destroyed or reduced the antibody-binding activity of the hexapeptide.

Further, a series of truncated peptides was made to analyse the immunoreactivity of the major EWVATISG epitope in the H chain (Fig 4B). The unabsorbed antiserum reacted weakly with the Glu-Trp dipeptide, and as the sequence was extended from the carboxy terminus, so the intensity of the reactions increased with the octapeptide EWVATISG showing the strongest reaction. However, when the amino terminal Glu was omitted, then antibodies did not react with WVATISG. In turn, the omission of the terminal Trp produced a peptide VATISG with which the serum again reacted strongly. In this particular peptide, the terminal Val was important because ATISG was only weakly reactive.

From this it appears that the EWVATISG contains two epitopes defined by Glu-Trp-Val--- with an exposed acetylated amino terminus, and ---Thr-Ile--- held in a favoured confor-

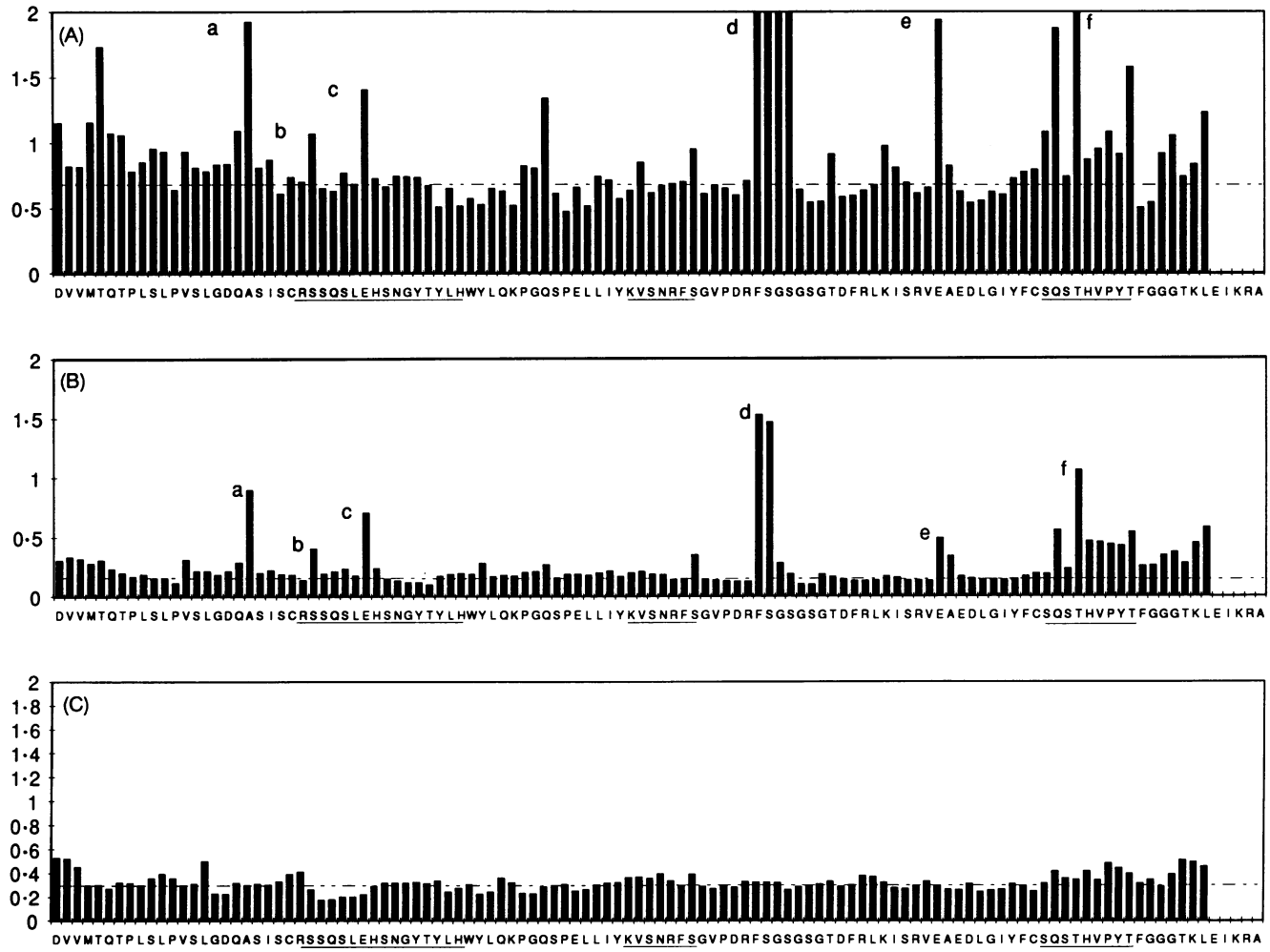

Figure 3. The idiotopes of the light chain of mAb V-88 identified by the rabbit anti-idiotype antiserum. Details of assay conditions and explanation of the results as in Fig. 2. The anti-V-88 antiserum was tested before (A) and after absorption (B), and compared with normal rabbit serum (C). The major idiotopes in the scan are lettered: (a) ASISCR, (b) SSQSLE, (c) EHSNGY, (d) FSGSGSG, (e) EAEDLG, (f) THVPYT.

mation by the flanking residues. Alternatively, the Glu may be unimportant, and the Trp obstructive when at the amino terminus, thus shielding the Thr-Ile epitope.

DISCUSSION

Through applying epitope scanning methods to the analysis of antisera raised against DNA-binding mAb derived from lupus mice, we have shown that they contain antibodies that react with linear peptide sequences in the V regions of the antibody H and L chains. These epitopes can be considered to comprise the continuous idiotopes of antibody V-88 which we assume also has discontinuous idiotopes (cf. ref. 1) that are undetectable here. The results show how polyclonal anti-Id reagents contain antibodies against many different epitopes. This explains the extensive cross-reactions such reagents have with panels of mAb[9] and also why anti-Id antisera raised and absorbed ostensibly in the same way may have quite different fine specificities.[25] Clearly, polyclonal anti-Id antibodies identify more components of the Id than can be defined with a monoclonal anti-Id antibody.

It was interesting to find that the continuous idiotopes of mAb V-88 were not restricted to the hypervariable regions of the antibody. The antigen-binding sites or paratopes of antibodies are defined functionally and they do not have physically defined boundaries. These results accord with the X-ray analysis of anti-lysozyme antibody–anti-Id crystals[1] and demonstrate that paratope (*viz.* specificity) and idiotype are not identical. It is clearly shown here that the Id of antibody V-88 extends beyond its likely paratope, an expected observation since not all anti-Id antibodies compete with antigen for binding to antibody. Certainly, some idiotopes were clearly located in CDR sequences, but others overlapped into the FW regions and yet others were entirely defined within FW sequences.

It was notable that the idiotopically active region in FW3 (residues 73–96) of the H chain is exposed on the external β-strands of the V_H region. In an intact antibody, this sequence encompasses a fourth (variable) loop (residues 84–91) that is not thought to contribute normally to binding antigen, but which may be involved in interactions with oligonucleotides.[26] The other major idiotopes are also at least partly exposed on external surfaces of the V_H/V_L dimer, making them accessible to anti-Id

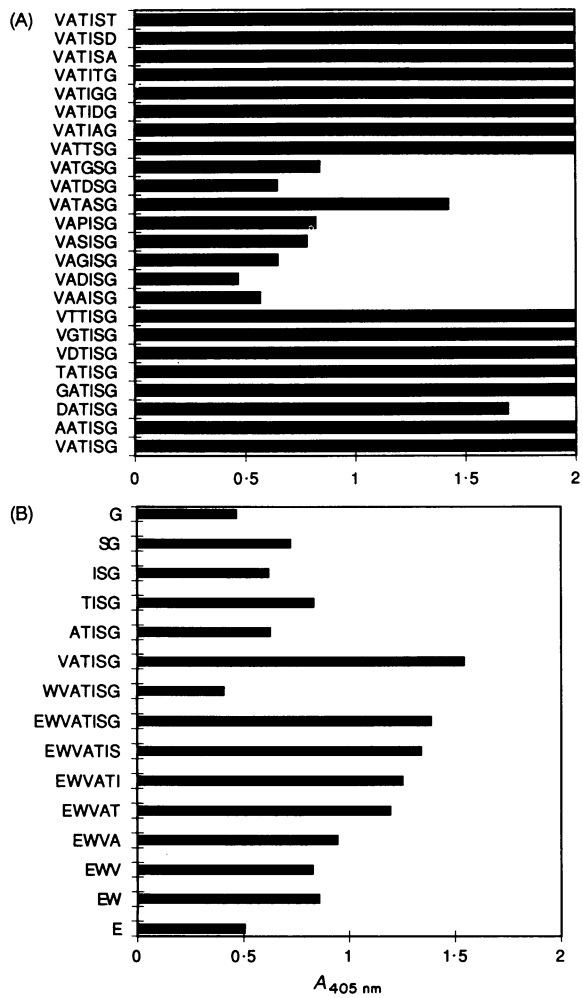

Figure 4. Reactivity of the unabsorbed rabbit anti-V-88 anti-idiotype serum with peptides corresponding to the dominant V_H idiotype. Substitution homologues of the hexapeptide VATISG (A) and degradates of the octopeptide EWVATISG (B) were synthesized and used exactly as described in the legend to Fig. 2.

antibodies in the intact molecule. A detailed modelling of the location of these idiotopes will be published separately.

The FW3 region of the L chain, idiotypically active in these experiments, has been identified as an area involved in the functional activity of some rheumatoid factors, in that it has been shown by Hay *et al.*,[27] using synthetic V_L region peptides as here, to participate in the binding of aggregated IgG. Some, but not all, activity of the rabbit anti-88 antiserum against this region was removed by absorption of the anti-serum on normal IgG, and we cannot exclude that (mouse) FW3 peptides captured rabbit Ig irrespective of its specificity. In the same way, self-binding interactions of some antibodies have been shown to depend upon V_H structures in the CDR2/FW3 area:[28] anti-88 antibodies reactive with sequences in this region were also removed by absorption.

The location of the 16/6 idiotype is suggested by this study. There are a number of cases where homologues of this sequence occur in other antibodies; as described, EWVATISG in FW2/CDR2 probably contained two epitopes in our scanning experiments, and is represented in other DNA-reactive lupus autoantibodies. The peptide sequence in HFW3 (73-96) is 80%

Table 1. Sequence homologies* of the FW2/CDR2 region of the mAb V-88 heavy chain

Id.16/6$^+$ monoclonal antibodies											
				<FW2|CDR2>							
L	E	W	V	A	T	I	S	G	D	V_H88	Mouse
-	-	-	-	S	A	-	-	-	S	V_H18/2	Human
-	-	-	-	S	A	-	-	D	S	V_HA73	Human
-	-	-	-	S	A	I	-	P	S	V_HA85	Human
-	-	-	-	S	S	-	-	-	S	V_HGL18	Human
-	-	-	-	S	V	-	Y	.	I	V_HA57	Human
hsp60											
I	A	Q	-	-	-	-	-	A	M	Rat/Chinese hamster (GroEL)	
I	A	Q	-	-	-	-	-	A	N	Human	
I	A	Q	-	-	S	-	-	A	N	*B. burgdorferi*	
I	A	Q	-	-	-	-	-	A	N	*C. psittaci* (HypB)	
I	A	Q	-	-	-	-	-	A	N	*S. cerevisiae*	
I	A	Q	-	G	-	-	-	A	N	*E. coli*	
I	A	Q	-	G	-	-	-	A	N	*C. burnetti* (HtpB)	
I	A	A	T	-	A	-	-	A	G	*M. bovis/M. tuberculosus*	
Other molecules											
P	C	S	H	-	-	-	-	-	N	TcR Vδ 3 human	
E	L	N	A	-	-	-	-	-	F	CD3γ human	

* Sources of sequences are given in the text.

(-) indicates identity, and (.) indicates gap introduced to achieve maximum sequence homology.

homologous to the corresponding sequence of antibody 18/2, encoded by the human *V_H26c* gene, which is Id.16/6$^+$.[29] As shown in Table 1, the FW2/CDR2 sequence of the V-88 H chain is homologous with several human antibodies expressing Id.16/6.[30] Because mAb V-88 also expresses Id.16/6,[10] some or all of these may represent parts of the 16/6 idiotype; the sequence LEWV--IS- is one candidate sequence for a 16/6 idiotope.

Homologues of the VATIS coding sequence can be found elsewhere, notably in heat-shock proteins (hsp) such as GroEL, the hsp60 of the rat, human and Chinese hamster (see ref. 31), and in *Chlamydia trachomatis* and *C. psittaci*,[32] and as VASIS in hsp65 of *Borelia bergdorferi*,[33] as VGTIS in hsp65 of *Escherichia coli*.[34] In all these, as shown in Table 1, the sequence is flanked by Ala at the carboxy terminus, which we found to be an acceptable substitution for the Gly of the V-88 sequence. This suggests a relationship between hsp and the autoantibody V-88 such that some anti-Id antibodies against V-88 are also antibodies against bacterial or autologous hsp which we know are immune targets in autoimmune rheumatic diseases.[35,36]

Thus an immune response to a hsp would involve the production of antibodies that, through idiotypic linkage, could stimulate the production of autoantibodies. As a corollary, an autoantigen-driven antibody response could itself prime for an anti-hsp response. Some of the organisms whose hsp show this homology are implicated in the aetiology of autoimmune connective diseases, which are also characterized by the production of both DNA autoantibodies and antibodies expressing the 16/6 idiotype.[37]

One implication of this model is that the rabbit that produced the anti-Id antiserum analysed here may have made some of the antibodies in response to the mycobacterial hsp in

the CFA. It is improbable that all the antibodies detected were made in response to such a stimulus because the sequence homologies for this are not documented. With regard to the hsp60 homologous sequence, the anti-Id.V-88 antiserum does react with peptides based on the relevant mycobacterial sequence (cf. Table 1). Further, rabbits immunized with other DNA mAb in CFA do not make antibodies against the VATISG sequence; antibodies against this sequence can be found in humans and mice who have not been (knowingly) exposed to mycobacteria, and who have lupus disease. These findings will be published elsewhere, and they support the conclusion that, in the serum studied here, the antibodies were made in response to the mAb used to immunize the rabbit.

The occurrence of homologues of sequences of antibody V regions in non-antibody molecules has been recorded in at least two other situations besides the hsp cases here. C-reactive protein contains a peptide motif also found in T15$^+$ anti-phosphorylcholine antibodies,[2] and Pucetti *et al.*[38] identified a sequence of RNP in the light chain of an antibody that was itself anti-idiotypic to an anti-RNP antibody. Other homologues of the VATISG sequence have been found in T-cell receptor (TcR) sequences. ATISG is a common sequence in members of the V$\alpha\delta$ subgroup 3 family,[39] and is also found in the trans-membrane portion of the CD3 γ-chain.[40] The significance of these homologies (Table 1) to the antibody sequence is unknown.

The fact that anti-Id antibodies react with short linear peptides may mean that some anti-Id antibodies are made against degraded antibody peptides in the first place. Several studies have shown that synthetic peptides corresponding to short V region sequences will induce anti-Id antibodies (refs 41,42; B. H. Hahn, personal communication). Rats immunized with a peptide corresponding to the LCDR1 sequence of V-88 make antibodies reactive with mAb V-88 itself and with two idiotopes in LCDR1 identified by scanning with the hexapeptides (N. A. Staines *et al.*, manuscript in preparation). It seems that there are not stringent size requirements for V region peptides to be immunogenic.

These studies collectively suggest that synthetic peptides are analogues of idiotopes and may be used to mimic their properties. Responses against them could be focused to induce high-affinity antibodies, and thus they may have distinct advantages over whole antibodies or anti-Id antisera/antibodies which generally appear to have limited powers to ameliorate lupus disease.[12,43–46] However, the mechanisms whereby anti-Id antibodies are induced against degraded antibody peptides are not explained. It is easy to understand that degradation of antibodies by antigen-processing cells will take place following intentional immunization, and it is assumed that this is also a natural process that would account for the existence of anti-Id antibodies produced in the course of an autoimmune disease. The role of the B cell in processing its own Id$^+$ receptor antibody, and endocytosed complementary anti-Id antibody, is especially attractive. It might be assumed that B cells presenting such degraded idiotype peptides would stimulate both T cells and B cells.

ACKNOWLEDGMENTS

The authors acknowledge with thanks the following support: a scholarship from the European Molecular Biology Organization to D.E., an SERC CASE award with the generous co-operation of The Boots Company, Nottingham to A.N.D., a grant from Lupus U.K. to F.J.W. and a Vacation Studentship from The Wellcome Trust to J.M.

REFERENCES

1. Bentley G.A., Boulot G., Riottot M.M. & Poljak R.J. (1990) Three-dimensional structure of an idiotype-anti-idiotype complex. *Nature*, **348,** 254.
2. Swanson S.J., Lin B.-F., Mullenix M.C. & Mortensen R.F. (1991) A synthetic peptide corresponding to the phosphorylcholine (PC)-binding region of human C-reactive protein possesses the TEPC-15 myeloma PC-idiotype. *J. Immunol.* **146,** 1596.
3. Watts R. & Isenberg D.A. (1990) DNA antibody idiotypes: an analysis of their disease connections and origins. *Int. Rev. Immunol.* **5,** 279.
4. Stollar B.D. (1981) Anti-DNA Antibodies. *Clin. Immunol. Allergy*, **1,** 243.
5. Marion T.N., Lawton A.R., Kearney J.F. & Briles D.E. (1982) Anti-DNA autoantibodies in (NZB × NZW)F_1 mice are clonally heterogeneous, but the majority share a common idiotype. *J. Immunol.* **128,** 668.
6. Morgan A., Buchanan R.R.C., Lew A.M., Olsen I. & Staines N.A. (1985) Five groups of antigenic determinants on DNA identified by monoclonal antibodies from (NZB × NZW)F_1 and MRL/lpr/lpr mice. *Immunology*, **55,** 75.
7. Isenberg D., Williams W., Axford J., Bakimer R., Bell D., Casasceca-Grayson T. *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.
8. Staines N.A., Thompson H.S.G. & Morgan A. (1985) Diversity and multispecificity of autoantibodies reactive will DNA: some evolutionary implications. *Protides Biol. Fluids.*, **33,** 241.
9. Staines N.A., Ravirajan C.T., Morgan A., Belcher A.J., Henry A.S., Lake R. *et al.* (1993) Expression and relationships of seven public idiotypes of DNA-binding autoantibodies on monoclonal antibodies and serum immunoglobulins. *Lupus* (in press).
10. Morgan A., Isenberg D.A., Naparstek Y., Rauch J., Duggan D., Khiroya R., Staines N.A. & Schattner A. (1985) Shared idiotypes are expressed on mouse and human anti-DNA autoantibodies. *Immunology*, **56,** 393.
11. Watts R.A., Ravirajan C.T., Staines N.A. & Isenberg D.A. (1991) Detection of human and murine public DNA antibody idiotypes in tissues and sera of patients with autoimmune diseases. *Clin. exp. Immunol.* **83,** 267.
12. Ravirajan C.T. & Staines N.A. (1991) Involvement in lupus disease of idiotypes Id.F-423 and Id.IV-228 defined, respectively, upon foetal and adult MRL/Mp/*lpr*/*lpr* DNA-binding monoclonal autoantibodies. *Immunology*, **74,** 342.
13. Geysen H.M., Rodda S.J., Mason T.J., Tribbick G. & Schoofs P.G. (1987) Strategies for epitope analysis using peptide synthesis. *J. immunol. Meth.* **102,** 259.
14. Eilat D., Webster D.M. & Rees A.R. (1988) V region sequences of anti-DNA and anti-RNA autoantibodies from NZB/NZW F_1 mice. *J. Immunol.* **141,** 1745.
15. Caton A.J., Brownlee G.G., Staudt L.M. & Gerhardt W. (1986) Structural and functional implications of a restricted antibody response to a defined antigenic region on the influenza virus hemagglutinin. *Eur. mol. Biol. Org. J.* **5,** 1577.
16. Brodeur P. & Riblet R. (1984) The immunoglobulin heavy chain variable region in the mouse. I. 100 IgH-V genes comprise 7 families of homologous genes. *Eur. J. Immunol.* **14,** 922.
17. Hartman B. & Rudikoff S. (1984) V_H genes encoding the immune response to beta-(1,6)-galactan: somatic mutation in IgM molecules. *Eur. mol. Biol. Org. J.* **3,** 3023.
18. Shlomchik M., Mascelli M., Shan H., Radic M.Z., Pisetsky D., Marshak-Rothstein A. & Weigert M. (1990) Anti-DNA anti-

bodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. exp. Med.* **171,** 265.

19. Marion T.N., Tillman D.M. & Jou N.T. (1990) Interclonal and intraclonal diversity among anti-DNA antibodies from an (NZB × NZW)F1 mouse. *J. Immunol.* **145,** 2322.
20. Potter M., Newell J.B., Rudikoff S. & Haber E. (1982) Classification of mouse VK groups based on the partial amino acid sequence to the first invariant tryptophan: impact of 14 new sequences from IgG myeloma proteins. *Molec. Immunol.* **19,** 1619.
21. Corbet S., Milili M., Fougerau M. & Schiff C. (1987) Two VK germline genes related to the GAT idiotypic network (Ab1 and Ab3/Ab1′) account for the major subfamilies of the mouse VK-1 variability subgroup. *J. Immunol.* **138,** 932.
22. Eilat D., Hochberg M., Tron F., Jacob L. & Bach J.-F. (1989) The VH gene sequences of anti-DNA antibodies in two different strains of lupus-prone mice are highly related. *Eur. J. Immunol.* **19,** 1241.
23. Kofler R., Strohal R., Balderas R.S., Johnson M.E., Noonan D.J., Duchosal M.A., Dixon F.J. & Theofilopolous A.N. (1988) Immunoglobulin κ light chain variable region gene complex organization and immunoglobulin genes encoding anti-DNA autoantibodies in lupus mice. *J. clin. Invest.* **82,** 852.
24. Hopp T.P. & Woods K.R. (1981) Prediction of protein antigenic determinants from amino acid sequences. *Proc. natl. Acad. Sci. U.S.A.* **78,** 3824.
25. Watts R.A., Hillson J., Oppliger I.R., MacKenzie L., Lydyard P.M., Mackworth Young C., Brown C., Staines N.A. & Isenberg D.A. (1992) Sequence analysis and idiotypic relationships of BEG-2, a human fetal antibody reactive with DNA. *Lupus*, **1,** 9.
26. Herron J.N., He X.M., Ballard D.W., Blier P.R., Pace P.E., Bothwell A.L.M., Voss E.W. & Edmundson A.B. (1991) An autoantibody to single-stranded DNA: comparison of the three-dimensional structures of the unligated Fab and a deoxynucleotide-Fab complex. *Proteins: Structure, Function Genet.* **11,** 159.
27. Hay F.C., Soltys A.J., Tribbick G. & Geysen H.M. (1991) Framework peptides from κIIIb rheumatoid factor light chains with binding activity for aggregated IgG. *Eur. J. Immunol.* **21,** 1837.
28. Kaveri S.-V., Halpern R., Kang C.-Y. & Kohler H. (1991) Antibodies of different specificities are self-binding: implication for antibody diversity. *Molec. Immunol.* **28,** 773.
29. Chen P., Liu M., Sinha S. & Carson D.A. (1988) A 16/6 idiotype-positive anti-DNA antibody is encoded by a conserved VH gene with no somatic mutations. *Arthritis Rheum.* **31,** 1429.
30. Young F., Tucker L., Rubinstein D., Guillaume T., André-Schwartz J., Barrett K.J., Schwartz R.S. & Logtenberg T. (1990) Molecular analysis of a germ-line-encoded idiotypic marker of pathogenic human lupus autoantibodies. *J. Immunol.* **145,** 2545.
31. Venner T.J. & Gupta R.S. (1990) Nucleotide sequence of rat hsp60 (chaperonin, GroEL homolog) cDNA. *Nucleic Acids Res.* **18,** 5309.
32. Morrison R.P., Belland R.J., Lyng K. & Caldwell H.D. (1989) Chlamydial disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J. exp. Med.* **170,** 1271.
33. Shanafelt M.-C., Hindersson P., Soderberg C., Mensi N., Turck C.W., Webb D., Yssel H. & Peltz G. (1991) T cell and antibody reactivity with the *Borelia burgdorferi* 60-kDa heat shock protein in Lyme Arthritis. *J. Immunol.* **146,** 3985.
34. Hemmingsen S.M., Woolford C., van der Vies S.M., Tilly K., Dennis D.T., Georgopolous C.P., Hendrix R.W. & Ellis R.J. (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. *Nature*, **333,** 330.
35. Dhillon V., Latchman D. & Isenberg D.A. (1991) Review. Heat shock proteins and systemic lupus erythematosus. *Lupus*, **1,** 3.
36. van Eden W., Hogervorst E.J.M., Wauben M.H.M., van der Zee R. & Boog C.J.P. (1991) Heat shock proteins in autoimmunity. *Biochem. Soc. Trans.* **19,** 171.
37. Isenberg D.A. & Staines N.A. (1990) Idiotypes of DNA-binding antibodies: analysis of their role in health and disease. *J. Autoimmunity*, **3,** 339.
38. Pucetti A., Koizumi T., Migliorini P., Andre-Schwartz J., Barret K.J. & Schwartz R.S. (1990) An immunoglobulin light chain from a lupus-prone mouse induces autoantibodies in normal mice. *J. exp. Med.* **171,** 1919.
39. Chothia C., Boswell D.R. & Lesk A. (1988) The outline structure of the T-cell alpha beta receptor. *Eur. mol. Biol. Org. J.* **7,** 3745.
40. Krissansen G.W., Owen M.J., Verbi W. & Crumpton M.J. (1986) Primary structure of the T3 γ subunit of the T3/T cell antigen receptor complex deduced from cDNA sequences: evolution of the T3 γ and δ subunits. *Eur. mol. Biol. Org. J.* **5,** 1799.
41. Meek K., Takei M., Dang H., Sanz I., Dauphinee M.J., Capra J.D. & Talal N. (1990) Anti-peptide antibodies detect a lupus-related interspecies idiotype that maps to H chain CDR2. *J. Immunol.* **144,** 1375.
42. Mazza G., Guigou V., Moinier D., Corbet S., Ollier P. & Fougereau M. (1987) Molecular interactions in the 'GAT' idiotypic network. An approach using synthetic peptides. *Ann. Inst. Pasteur/Immunol. Paris*, **136D,** 259.
43. Hahn B.H. & Ebling F.M. (1984) Suppression of murine lupus nephritis by administration of an anti-idiotypic antibody to anti-DNA. *J. Immunol.* **132,** 187.
44. Mahana W., Guilbert B. & Avrameas S. (1987) Suppression of anti-DNA antibody production in MRL mice by treatment with anti-idiotypic antibodies. *Clin. exp. Immunol.* **70,** 538.
45. Epstein A., Greenberg M., Diamond B. & Grayzel A.I. (1987) Suppression of anti-DNA antibody synthesis *in vitro* by a cross-reactive antiidiotypic antibody. *J. clin. Invest.* **79,** 997.
46. Morland C., Michael J., Adu D., Kizaki T., Howie A., Morgan A. & Staines N.A. (1991) Anti-idiotype and immunosuppressant treatment of murine lupus. *Clin. exp. Immunol.* **83,** 126.