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Selective recognition of DNA antigenic determinants by murine monoclonal anti-DNA antibodies

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SUMMARY

To assess the immune recognition of DNA in systemic lupus erythematosus, the antigenic specificity of monoclonal anti-DNA antibodies from autoimmune MRL-*lpr/lpr* mice was investigated. Determinant specificity was assessed by ELISA in terms of binding to a panel of ssDNA antigens including calf thymus, human placenta, *Escherichia coli*, *Clostridium perfringens*, *Micrococcus lysodeikticus*, salmon testes, chicken blood and murine DNA. Among the monoclonal antibodies, a variety of binding patterns was observed, although for all antibodies tested murine DNA was among the most reactive antigens. Binding to other DNAs varied markedly, with some antibodies showing only low reactivity to certain antigens in the test panel. Similar results were obtained with sera of individual MRL-*lpr/lpr* mice. These results suggest that anti-DNA antibodies bind specific antigenic determinants variably expressed by DNAs of various species. Furthermore, the preferential binding to mouse DNA by some MRL-*lpr/lpr* antibodies may suggest a role of self-DNA in the *in vivo* selection of anti-DNA antibodies for expression.

Keywords anti-DNA antibodies DNA antigenicity monoclonal antibodies systemic lupus erythematosus murine models of autoimmunity

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-system inflammatory disease characterized by the production of antibodies to DNA. These antibodies serve as markers of diagnostic and prognostic significance, with anti-DNA levels frequently correlating with measures of clinical disease activity, particularly nephritis. These associations, along with the finding of anti-DNA antibodies in immunoglobulin deposits in renal lesions, have implicated anti-DNA antibodies in the pathogenesis of SLE (Tan, 1982; Emlen, Pisetsky & Taylor, 1986; Stollar, 1986). Because of the central role of anti-DNA in SLE, the cellular basis of this response has been intensively investigated, evaluating in particular the respective roles of polyclonal B cell activation and antigen-specific drive (Klinman & Steinberg, 1987; Gharavi & Elkon, 1988). Although the detailed mechanisms of this response remain unknown, recent studies on anti-DNA variable (V) regions genes have suggested that DNA antigen specifically stimulates this response. Thus, monoclonal anti-DNA antibodies from lupus mice display sequences and patterns of somatic mutation consistent with *in vivo* selection by DNA (Shlomchik *et al.*, 1987; Marion *et al.*, 1989).

In a previous analysis of monoclonal anti-DNA antibodies from autoimmune MRL-*lpr/lpr* mice, our laboratory demonstrated differential antibody binding to a panel of DNAs of various species origin (Karounos & Pisetsky, 1987). These patterns suggested selective recognition of DNA sites variably expressed on different DNAs, although this panel did not include self-DNA. We have, therefore, extended this analysis to additional monoclonal anti-DNAs and expanded the antigen panel to include murine as well as other species of DNA. Here we confirm that murine monoclonal anti-DNA antibodies display variable reactivity to DNA from different species and demonstrate, moreover, that mouse DNA is frequently a preferred antigen. Similar results were obtained with sera. These findings suggest that anti-DNA antibodies selectively recognize DNA antigenic determinants variably expressed on different DNAs. Furthermore, the preferential reactivity to murine DNA may be evidence for a role of self-DNA in the *in vivo* selection of anti-DNA antibodies for expression.

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MATERIALS AND METHODS

Antigens

Calf thymus (CT), human placenta (HP), *Escherichia coli* (EC), *Clostridium perfringens* (CP), *Micrococcus lysodeikticus* (MC), and salmon testes (ST) DNA were purchased from Sigma Chemical Co. (St Louis, MO); chicken blood (CB) DNA, from PL Biochemicals (Milwaukee, WI); and MRL-*lpr/lpr* DNA, from the Jackson Laboratory (Bar Harbor, ME). Each DNA

Table 1. Isotype analysis of monoclonal anti-DNA antibodies

Antibody	Isotype
F1/SS1	IgM κ
F1/SS12	IgM κ
F1/SS7	IgG3 κ
F1/N1	IgG1 κ
F1/SS9	IgG1 κ
6/0	IgG2a κ

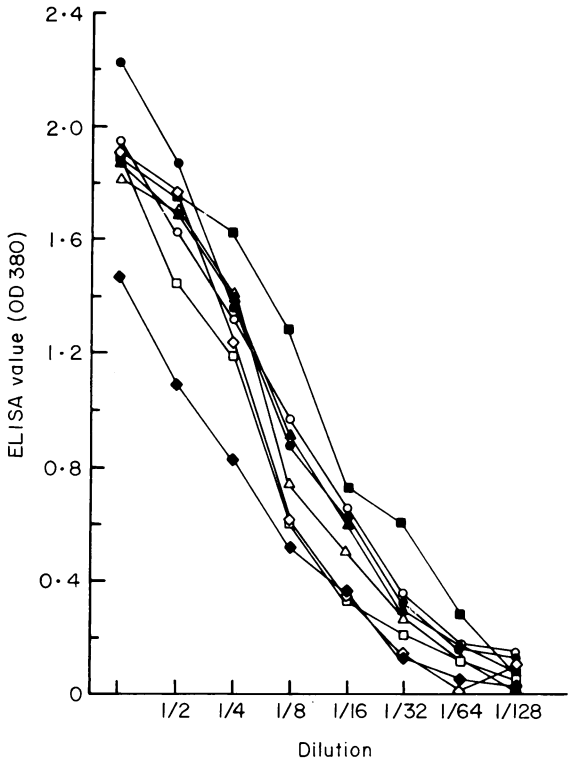

Fig. 1. Specificity analysis of monoclonal antibody F1/SS1. Binding activity of antibody F1/SS1 was determined by ELISA using as antigens the following DNAs: MRL (closed circles); *Clostridium perfringens* (open circles); calf-thymus (closed squares); *Escherichia coli* (closed triangles); chicken blood (open triangles); human placenta (open squares); salmon testes (open diamonds); and *Micrococcus lysodeikticus* (closed diamonds). Starting concentration of antibody was 5 μg/ml.

preparation was extracted with phenol, followed by isoamyl alcohol/chloroform (24:1) to remove protein contamination and then precipitated by 95% ethanol. The DNA was redissolved in SSC buffer (0·1 M NaCl, 0·015 M Na citrate, pH 8·0), boiled for 10 min and then rapidly immersed in ice to produce ssDNA. The concentration of DNA was determined by absorbance at 260 nm. Protein contamination was assessed by optical density (OD) 260/280 ratios with all DNAs used in these experiments having ratios > 1·9.

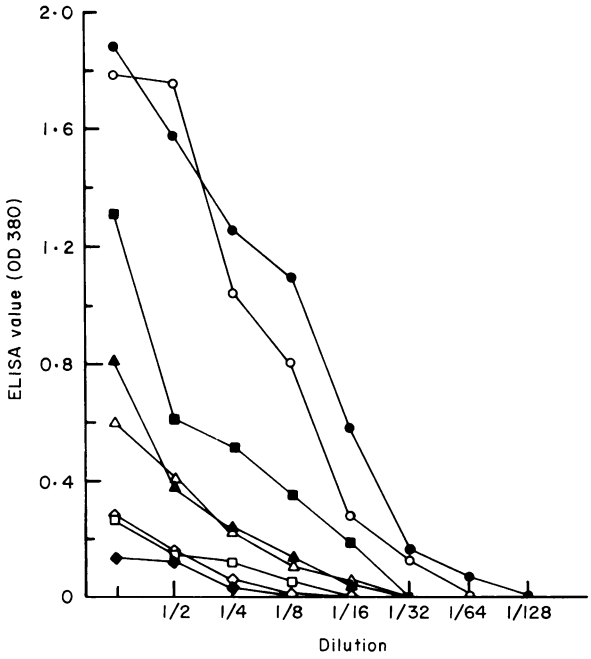

Fig. 2. Specificity analysis of monoclonal antibody 6/0. Binding activity of antibody was tested as described in Fig. 1. Starting concentration of antibody was 6 μg/ml.

Monoclonal antibodies and sera

Monoclonal anti-DNA antibodies in this study were obtained by standard techniques from spleen cells of MRL-*lpr*/*lpr* mice using the NS1 cell line as fusion partner. All hybrid cell lines were cloned twice by limiting dilution and monoclonality demonstrated by subclass analysis. Preparations used in these studies were obtained from tissue culture supernatants and purified initially by precipitation with 50% ammonium sulphate. The precipitates were then dissolved in phosphate-buffered saline (PBS) and dialysed overnight against PBS. The antibodies were further purified on Sepharose–Protein A or Sepharose–rabbit anti-mouse immunoglobulin columns. MRL-*lpr*/*lpr* sera were obtained from male mice purchased from the Jackson Laboratory and raised under conventional conditions in the animal facilities of the Durham VA Hospital.

Antibody binding assays

Direct binding assays were performed by ELISA, as previously described (Karounos & Pisetsky, 1987). Briefly, 96-well polystyrene microtitre plates (Dynatech Laboratories, Alexandria, VA) were coated with ssDNA at a concentration of 5 μg/ml and incubated at 4 °C overnight. The plates were washed with PBS containing 0·05% Tween 20 (PBS-T); serial dilutions of the monoclonal anti-DNA antibodies in PBS-T were then added. After 45 min, the plates were washed with PBS-T and then incubated with a 1:6000 dilution of a peroxidase-conjugated goat anti-mouse immunoglobulin reagent (H + L chain specific; Organon Teknika, Durham, NC) in PBS-T. After 45-min incubation and washing, a substrate solution of 3,3′, 5,5′-tetramethylbenzidine (Organon Teknika) and 0·015% H_2O_2 in 0·1 M, citrate buffer, pH 4, was added. The absorbances were measured at 380 nm after a 30-min incubation using a Titertek Multiskan Spectrophotometer (Flow Laboratories, McLean,

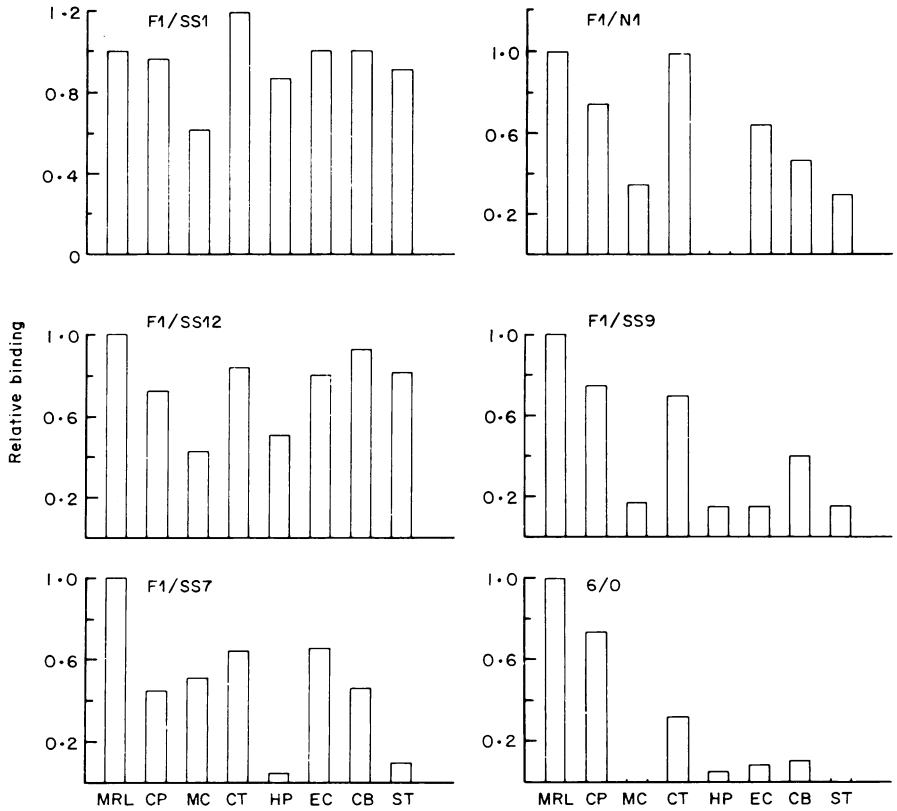

Fig. 3. Relative DNA binding of monoclonal anti-DNA antibodies. Relative activity of each monoclonal antibody for the different DNAs is presented using normalized values. In each case, MRL DNA is assigned a value of 1·0. CP, *Clostridium perfringens*; MC, *Micrococcus lysodeikticus*; CT, calf thymus; HP, human placenta; EC, *Escherichia coli*; CB, chicken blood; ST, Salmon testes.

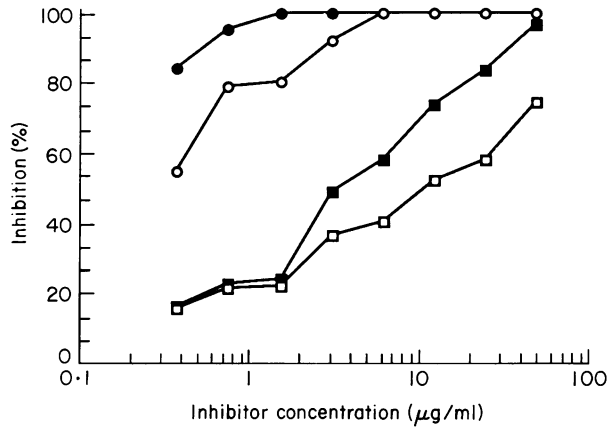

Fig. 4. Inhibition analysis of monoclonal antibody 6/0 binding. To determine the specificity for fluid-phase DNA, antibody 6/0 was incubated with varying concentrations of *Clostridium perfringens* (CP) (closed circles and squares) and human placenta (HP) (open circles and squares) DNA prior to assay for binding to ssCP (squares) or ssHP (circles) DNA.

VA). Inhibition assays were accomplished by incubating the monoclonal antibodies with varying concentrations of inhibiting DNAs. The concentrations of the monoclonal antibodies used were determined by prior titration to produce an OD at 380 nm (OD_{380}) value of approximately 1 on the coat DNA. After 45 min of incubation, the DNA/antibody mixture was added to coated plates. Assays were then performed as described for the standard ELISA. Values calculated were the percentage inhibition compared with the binding of the uninhibited antibody.

To facilitate comparison of the relative binding activities of the different monoclonal antibodies, a normalization procedure was used. First, a full titration of each antibody on the different DNAs was performed and a dilution established where the OD_{380} value for MRL DNA was approximately 1. This value was established as 1·0 and a conversion factor derived to adjust all other OD_{380} values at that same dilution. MRL DNA therefore produced a value of 1·0 for all antibodies tested although the actual specific activities (i.e. protein concentration producing an OD value of 1) may have differed among the antibodies.

RESULTS

The isotypes of the monoclonal antibodies used in this study are listed in Table 1; antibodies denoted F1 were all obtained from the same mouse and 6/0 from another. These antibodies had been originally identified using CT DNA as the screening antigen in an ELISA. Figs 1 and 2 present full titrations for antibodies F1/SS1 and 6/0 respectively. These data indicate that these antibodies can be distinguished in terms of their specificity for different DNA antigens, with F1/SS1 showing significant binding to all DNAs over a narrow range of reactivity. 6/0, in contrast, showed only low levels of binding (OD $<0·5$ at 6 μg/

Table 2. MRL-*lpr*/*lpr* sera binding of various DNA antigens

	Antigen							
Dilution	MRL	EC	CP	HP	CB	MC	ST	CT
1/100	1·606	1·210	1·895	1·225	1·337	0·833*	0·553*	1·768
	(0·206)	(0·303)	(0·239)	(0·306)	(0·343)	(0·255)	(0·109)	(0·250)
1/400	1·188	·855	1·563	0·707	0·877	0·378*	0·254*	1·338
	(0·391)	(0·559)	(0·279)	(0·319)	(0·290)	(0·218)	(0·115)	(0·286)
1/1600	0·574	0·290*	0·904	0·296*	0·372	0·135*	0·090*	0·696
	(0·146)	(0·162)	(0·164)	(0·129)	(0·174)	(0·083)	(0·037)	(0·271)

Mean (s.d.) for five individual mice.

* DNAs exhibiting significant difference in binding from MRL DNA at $P<0·05$ by two-tailed Mann Whitney *U*-test.

EC, *Escherichia coli*; CP, *Clostridium perfringens*; HP, human placenta; CB, chicken blood; MC, *Micrococcus lysodeikticus*; ST, salmon testes; CT, calf thymus.

ml) to three of the DNA antigens in the panel and a broader range of reactivities with the other DNAs. For both antibodies, MRL DNA produced antibody binding at least as strong as any other DNA tested.

Specificity analysis of this kind is based on similarity in the antigenic content of the DNAs coated under these conditions. This assumption was supported in previous studies on other monoclonal anti-DNAs and in the current studies (Karounos & Pisetsky, 1987; Gilkeson, Grudier & Pisetsky, 1989). Thus, some of the antibodies, e.g. F1/SS1, and others to be described showed similar binding to most of the DNAs in the panel. Furthermore, in confirmation of previous studies, some SLE sera bound all the DNAs to a similar extent (data not shown). These observations suggest that the low binding of some antibodies to certain DNAs does not reflect a deficiency of solid-phase DNA antigen.

Complete titrations were performed for all the monoclonal antibodies and results were normalized to facilitate comparison (Fig. 3). These data indicate a diversity of binding reactions for the antibodies in this set, with some antibodies binding all the DNAs well and others being more selective. It is again noteworthy that MRL DNA was the preferred antigen for almost all the antibodies, suggesting that self-DNA is enriched in antigenic sites bound by these monoclonal antibodies.

To determine whether antibodies bind selectively to fluid as well as solid-phase DNA, inhibition assays were performed testing the ability of fluid-phase DNA to inhibit antibody binding in the ELISA. Figure 4 presents data from experiments with antibody 6/0 which discriminates among DNA antigens; HP and CP DNA were tested as representative antigens. As these data indicate, CP DNA was a more effective inhibitor than HP DNA, irrespective of the solid-phase DNA antigen, consistent with preference of antibody 6/0 for solid-phase CP DNA. However, the concentration of DNA required to produce 50% inhibition differed markedly between the two DNA coats, with both DNAs producing more effective inhibition of 6/0 binding to HP DNA than CP DNA. These results suggest that the assessment of antibody binding by inhibition assays is affected by preferences of antibodies for solid- *versus* fluid-phase antigen as well as avidity differences not apparent using solid-phase antigens. Nevertheless, differences in antibody binding to soluble antigens can be demonstrated.

Sera of MRL-*lpr*/*lpr* mice were next analysed by the same methods to determine whether the antibodies expressed in disease also selectively recognized DNA antigen. Table 2 shows the mean responses of sera from five MRL-*lpr*/*lpr* mice at three dilutions. As these data demonstrate, sera varied in their binding to the different DNAs. For each serum, mouse DNA uniformly yielded high binding activities which were comparable to those of CP and CT DNA. Of the DNAs tested, MC and ST were the least antigenic. These studies confirm observations on the monoclonal antibodies and further support the notion that targeting of antigenic determinants on DNA is selective.

DISCUSSION

These studies demonstrate that murine anti-DNA antibodies bind selectively to naturally occurring DNAs with self-DNA uniformly a strong antigen. Selective DNA recognition can be inferred from studies demonstrating that monoclonal anti-DNA antibodies bind variably to synthetic polynucleotide antigens, suggesting specificity for structural determinants which require specific base sequences or conformations (Lafer *et al.*, 1981; Lee *et al.*, 1981; Shoenfeld *et al.*, 1983). Indeed, analysis of the binding of a monoclonal anti-DNA to a restriction digest of a plasmid DNA demonstrated highly selective recognition of different fragments, probably because of the presence of unique epitopes created by base sequence arrays (Impraim *et al.*, 1985). Such binding would differ from the simple recognition of a sugar–phosphate backbone, for example, which should produce a more generalized binding to DNA antigens, either natural or synthetic. It is likely, however, that the specificity of anti-DNA antibodies is diverse, and that conformational, sequential and backbone determinants can all be targets of anti-DNA recognition (Morgan *et al.*, 1985).

Only a few studies have used natural DNAs to analyse the specificity of anti-DNA antibodies. Stollar, Levine & Marmur (1962) tested SLE patient sera with a large panel of DNA antigens from bacteriophages, bacteria, plants, as well as mammals, but not human DNA. This analysis showed marked differences in the antigenicity of various DNAs. It is of interest that certain bacterial DNAs were more antigenic than either murine or calf thymus DNA, indicating as found in our study, that antigenicity is not directly correlated with species of origin

(e.g. mammalian *versus* non-mammalian). Furthermore, the antigenicity of DNA from various species of bacteria, e.g. *Bacillus* or *Clostridium* showed marked discrepancies, suggesting that limited sequence differences may determine antigenicity.

A role of DNA in the induction of anti-DNA antibodies also emerges from consideration of our data showing that murine DNA was a preferred antigen for monoclonal as well as serum antibodies. The simplest explanation of these findings is that the anti-DNA response is antigen-driven and that self-DNA is the selecting antigen. Since self-DNA is ubiquitous in the organism, it would be the most likely source of antigen to drive this response once tolerance is broken. The pattern of specificity we observed is less readily explained by anti-DNA induction by polyclonal B cell activation. Although the normal B cell repertoire contains abundant anti-DNA precursors that can be induced non-specifically, the specificity of such natural autoantibodies has been considered to be broad (Dighiero *et al.*, 1985). Indeed, these antibodies frequently display polyspecific interactions with many different antigens, both self and foreign, and this property might be a clue to their physiological role (Schwartz & Stollar, 1985; Naparstek *et al.*, 1986). Polyspecific antibodies would be expected to recognize commonly expressed structural determinants, e.g. repeated negative charge groups, present on DNAs as well as other antigens. The binding of specific DNA sequences or conformations would be contrary to this postulated specificity for natural autoantibodies. Since the natural autoantibodies have not been evaluated by our methods, we cannot, however, exclude an unexpected skewing of their specificity for self-DNA.

It is of interest in this regard that serum anti-DNAs recognized some bacterial DNAs as well as murine DNA. This result could be explained by fortuitous sharing of certain antigenic sites in murine and bacterial DNAs, in a distribution not directly related to species of origin. An alternative explanation for the antigenicity of bacterial DNAs emerges from recent observations from our laboratory on naturally occurring and induced responses to bacterial DNA. These studies have suggested that foreign DNA is ordinarily immunogenic (Gilkeson *et al.*, 1989) and may prime a cross-reactive anti-DNA response in SLE (Karounos, Grudier & Pisetsky, 1988). The high binding to antibodies to certain bacterial as well as murine DNA may therefore reflect a role of foreign DNA in stimulating anti-DNA production as a close molecular mimic of self-DNA. Studies are in progress to evaluate these mechanisms in mice by identifying the sequences in mammalian and bacterial DNA targeted by these antibodies.

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