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Anti-DNA Autoantibodies from an MRL/*lpr* Mouse

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Twenty-one monoclonal anti-DNA autoantibodies were produced by fusing spleen cells from an autoimmune MRL/*lpr* mouse with SP2/0 myeloma cells. Hybridomas generated by the fusions were chosen for cloning on the basis of DNA binding by supernatant antibody. Each monoclonal antibody was purified to homogeneity and analyzed for the heavy and light chain isotypes and the binding specificity for single-stranded DNA, double-stranded DNA, and RNA. Sequence specificities and isoelectric points of the antibodies were also examined. All of the antibodies were IgG and tended to bind to both single-stranded and double-stranded DNA with a preference for the double-stranded form. Some of them also bound to RNA. Isoelectric points of the antibodies were shown to be high. The antibodies described in this report have characteristics of pathogenic anti-DNA antibodies.

Systemic lupus erythematosus (SLE) is considered to be the prototype systemic autoimmune disease that has the potential to directly involve multiple organ systems (Vyse et al., 1996). An important characteristic of SLE anti-DNA antibodies is their ability to bind to both single-stranded (ss) and double-stranded (ds) DNA. The anti-DNA autoantibodies are prominent in the sera of patients with SLE and several strains of mice (MRL/*lpr*, NZB/NZW F1, and BXSB) that develop SLE-like autoimmune syndromes, and levels of these antibodies have diagnostic and prognostic significance (Shlomchik et al., 1987; Stollar 1994; Koren et al., 1995). These antibodies form immune complexes which are deposited in various tissues, especially in the kidneys and joints (Hentati et al., 1991). However, the mechanism by which autoantibodies mediate SLE pathology remains unclear.

Two kinds of anti-DNA autoantibodies have been identified: natural autoantibodies, IgM isotypes with low affinity for ssDNA; and pathogenic autoantibodies which are mainly IgG with higher affinity for native, dsDNA than for denatured, ssDNA (Stollar, 1994). Single-stranded DNA-binding natural autoantibodies occur in low concentrations in neonatal and normal sera and their production is increased by polyclonal B cell activators. Patients with SLE produce large amounts of autoantibodies to diverse nucleic acids: ssDNA, dsDNA, dsRNA, ribosomal RNA, Z-DNA, and poly (ADP-ribose). They also form antibodies against other nuclear antigens including histones, RNP, RNA polymerase, topoisomerase, tRNA synthase, and HMG proteins (Tan, 1989).

Because it has been known that the anti-dsDNA antibodies contribute to the development of lesions and clinical diseases, it is important to know how they interact with DNA and in some cases, with other cross-reacting antigens (Raz et al., 1993; Reichlin et al., 1994; Jang et al., 1996). Some autoantibodies to dsDNA can recognize base sequence information, probably through interactions in the major groove (Stollar et al., 1986; Jang and Stollar, 1990). Some autoantibodies specifically discriminate among poly-nucleotides of different poly(dA-dT) (Tron et al., 1980) or poly(dG-dC) (Hahn and Ebling, 1984) sequences. On the other hand, many antibodies against helical nucleic acids appear to recognize the overall shape or the outer features of the helix rather than the sequence. Thus, immunization-induced antibodies to dsRNA, RNA-DNA hybrids, or triple-helical DNA and some lupus antibodies against dsDNA react with corresponding helices of completely different base composition (Stollar, 1994). However, knowledge about the interaction of these autoantibodies with DNA is still unclear.

One of the interesting features of the anti-DNA antibodies that have been reported is the H chain dominance in binding to DNA (Jang et al., 1996, 1998; Radic et al., 1991; Barry and Lee, 1993; Polymenis and Stollar, 1995). Data from UV cross-linking experiments indicate preliminary evidence for the chain dominance (Jang and Stollar, 1990). However, the chain dominance has not been systematically analyzed with a number of antibodies although in several antibodies, the H chain dominance has been reported.

In this study, we obtained a large number of hybridoma autoantibodies reacting with nucleic acids

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from an MRL/*lpr* mouse, and analyzed the antigen-binding characteristics of these antibodies. The auto-antibodies have characteristics similar to those that are pathogenic because they bind to dsDNA with higher affinity than to ssDNA and have IgG isotypes and high isoelectric points. With these monocloanl anti-DNA antibodies, analysis of the chain dominance and the detailed structures of their genes will be performed.

Materials and Methods

Production of monoclonal antibodies

Monocional anti-DNA antibodies were obtained by fusing spleen cells from a 24 week-old MRL/*lpr* mouse with a myeloma cell line, SP2/0 by 50% polyethylene glycol (PEG) 4000 (mol wt 3,000-4,000) (GIBCO BRL) solution following the PEG method (Gefter et al., 1977). Hybridoma cells were maintained in a HAT medium (Littlefield, 1964) supplemented with 20% FBS and 100 μg/ml penicillin-streptomycin and grown at 37 ℃ in a humidified incubator supplied with 5% CO_2. Hybridoma culture supernatants were screened for antibodies to native and denatured calf thymus DNA (Sigma) by ELISA as described previously (Shoenfeld et al., 1982). Selected cells were cloned twice by limiting dilution with RPMI containing 10% FBS. The isotypes of the anti-DNA monoclonal antibodies were determined with the culture supernatant using an isotyping kit (Pierce).

Purification of monoclonal antibodies

The antibodies (Ab) were purified by affinity chromatography using a single strand DNA agarose (Gibco BRL) column. The Ab-bound ssDNA agarose column was washed with 10 volumes of PBS and the antibodies eluted with 700 mM NaCl. The eluted fractions were dialyzed in PBS. The concentrations of Abs were measured by absorbance at 280 nm using a spectrophotometer. Purified Abs were detected by 12% SDS-PAGE and western blotting.

Direct-binding ELISA

Ninety-six well polystyrene microtiter plates (Nunc) were incubated with DNA at a concentration of 1 μg/ml or with yeast RNA (USB) at 100 μg/ml overnight at 4 ℃. The plates were washed 3 times with PBS containing 0.05% Tween 20 (PBST) and blocked with 3% BSA-PBST for 2 h at room temperature (RT). Serial dilutions of the monoclonal antibodies in PBS were then added. After incubation for 1 h at RT, the plates were washed three times with PBST, then incubated with a 1:10,000 dilution of alkaline phosphatase-conjugated goat anti-mouse polyvalent Ig (Sigma) in 1% BSA-PBST for 2 h at RT. After five washes, a substrate solution of *p*-nitrophenyl phosphate (Sigma) was added. Absorbances were measured at 405 nm using an ELISA reader (Bio-Rad).

Competitive ELISA

Competitive assays were accomplished by incubating the monoclonal antibodies with varying concentrations of polynucleotides: poly(dA) · poly(dT), poly(dG) · poly (dC), poly(dA-dT) · poly(dA-dT), and poly(dG-dC) · poly (dG-dC). The concentrations of the monoclonal antibodies used in competitive ELISA were determined by prior titration to produce an OD value of 1 at 405 nm by direct-binding ELISA. After incubation, the polynucleotide/antibody mixtures were transfered to a dsDNA (1 μg/ml)-coated microtiter plate. Assays were then performed as described for direct-binding ELISA.

Table 1. Analysis of characteristics of monoclonal anti-DNA antibodies and their relative antigenic affinities and specificities

Name of clones	Isotypes		DNA binding specificity[a]		RNA binding specificity[b]
	H chain	L chain	ssDNA	dsDNA	
G1-2	IgG2a	κ	-	+	-
G1-5	IgG2a	κ	+	++	+
G1-21	IgG2a	κ	++	++++	+++
G2-6	IgG2a	κ	-	+	++
G2-12	IgG2a	κ	-	++	+
G2-13	IgG2a	κ	-	+	+
G3-47	IgG2a	κ	++++	+++	+
G4-2	IgG2a	κ	++++	++++	+++
G4-8	IgG2a	κ	-	+	++
G4-9	IgG2a	κ	++	++	+
G4-20	IgG2a	κ	+	+	+
G5-8	IgG2b	λ	+	+++	-
G5-14	IgG2b	λ	+	+++	-
G5-17	IgG2b	λ	++++	++++	-
G5-32	IgG2b	λ	+++	++++	-
G5-33	IgG2b	λ	++	+++	+
G6-7	IgG2b	λ	++++	++++	-
G6-9	IgG2b	λ	+++	++++	-
G6-20	IgG2b	λ	+	++	-
G6-22	IgG2b	λ	+	++	+
G6-23	IgG2b	λ	++++	++++	-

[a, b] The nucleic acid binding specificity is expressed on the basis of the difference in OD values compared to the negative control. When the OD value of antibody is less than 1.5 times to that of negative control, it is represented with -, whereas 1.6 to 4 times was marked with +, 4.1 to 8 times with ++, 8.1 to 10 times with +++, and more than 10.1 times with ++++.

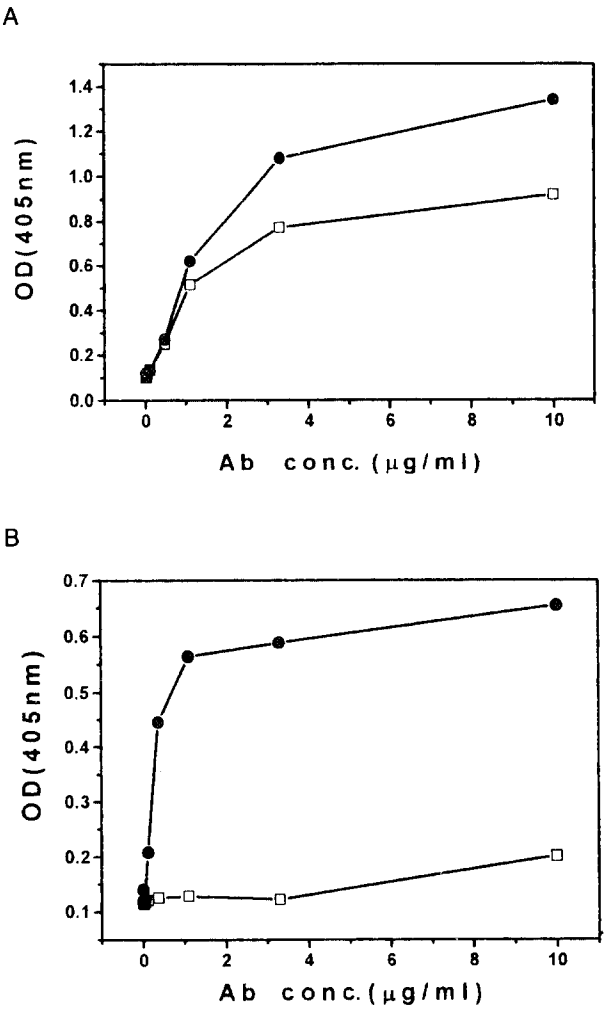

Fig. 1. Direct binding to ss- (□) and dsDNA (●) of two purified monoclonal antibodies, G4-2 (A) and G1-5 (B).

Results

Production of monoclonal antibodies and analysis of their antigenic specificities

Fusion of spleen cells from a single 24 week-old, unimmunized MRL/*lpr* mouse with SP2/0 myeloma cells yielded about 380 fused cultures. Hybridoma clones producing antibodies against DNA were selected by ELISA and subcloned twice. Twenty-one hybridoma clones producing anti-DNA monoclonal antibodies were obtained (Table 1). The isotypes of the H and L chains of the antibodies were analyzed by ELISA. One group of the clones had IgG2a and κ isotypes. The isotypes of the other group were IgG2b and λ. All of these antibodies had isoelectric points above 9.6 (data not shown).

To assess the conformational specificity of antibodies against single-stranded and double-stranded forms of DNA, we performed direct-binding ELISA. Most of these antibodies bound to dsDNA more strongly than to ssDNA (Table 1). Some of them bound well to both forms. However, the relative binding affinity to ss- and dsDNA varied depending on the type of antibody. For example, as shown in Fig. 1, the difference in the binding affinity to ss- and dsDNA of G4-2 was relatively small, whereas the binding affinity of G1-5 to dsDNA was much higher than that to ssDNA.

To analyze whether or not these anti-DNA antibodies have cross-reactivity with RNA, direct-binding ELISA was performed. Four clones of antibodies showed cross-reactivity with RNA with a relatively high affinity, whereas eight other clones bound to RNA with mild affinity (Table 1).

Table 2. The most preferred sequences for binding by monoclonal anti-DNA antibodies

Name of clones	Polynucleotides
G1-2	A · T[a]
G1-5	A · T
G1-21	A · T
G2-6	A · T
G2-12	A · T
G2-13	A · T
G3-47	AT · AT[b]
G4-2	A · T
G4-8	A · T
G4-9	A · T
G4-20	A · T
G5-8	AT · AT
G5-14	GC · GC[c]
G5-17	AT · AT
G5-32	A · T
G5-33	A · T
G6-7	GC · GC
G6-9	AT · AT
G6-20	AT · AT
G6-22	GC · GC
G6-23	GC · GC

[a](dA)n · (dT)n, [b](dA-dT)n · (dA-dT)n, [c](dG-dC)n · (dG-dC)n

Sequence specificities of monoclonal antibodies

To analyze sequence specificities of the antibodies for DNA binding, we performed competitive ELISA. Each antibody was tested with various concentrations of 4 polynucleotides, poly(dA) · poly(dT), poly(dG) · poly(dC), poly(dA-dT) · poly(dA-dT), and poly(dG-dC) · poly(dG-dC). The most preferred sequence of each antibody is shown in Table 2. All of them showed broad cross-reactivity with all sequences, although there were different patterns of inhibition by the polynucleotide sequences depending on the antibodies (data not shown). Fig. 2 shows the inhibition of DNA binding to the antibody, G4-2, by several polynucleotides.

Discussion

Since MRL/*lpr* mice were first identified in 1977, it was observed to develop massive lymphadenopathy, splenomegaly, hypergammaglobulinemia, and serum autoantibodies. They have been widely used as a model in the study of human SLE (Shlomchik et al., 1994). They develop generalized autoimmune diseases

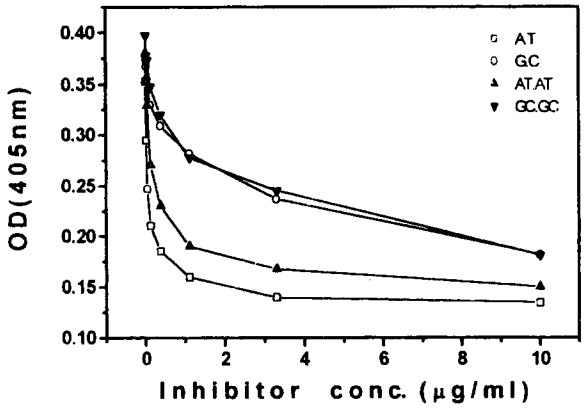

Fig. 2. Inhibition of a complex formation between anti-DNA antibody, G4-2 and dsDNA by different polynucleotides. A · T, (dA)n · (dT)n; G · C, (dG)n · (dC)n; AT · AT, (dA-dT)n · (dA-dT)n; GC · GC, (dG-dC)n · (dG-dC)n.

including glomerulonephritis, arthritis, and immune complex disease by 8 wk of age and die by 17-24 weeks of age (Smith and Steinberg, 1983; Moyer et al., 1987; Tan, 1989).

In the previous experiments, we observed that 24 wk-old MRL/*lpr* mice produced a higher titer of anti-DNA antibodies in their sera compared to 6 wk-old mice (Park et al., 1997). In these experiments, a number of anti-DNA hybridoma autoantibodies were produced from a 24 wk-old MRL/*lpr* mouse. The antibodies have characteristics of pathogenic anti-DNA antibodies.

Purine or pyrimidine bases, nucleosides, or mononucleotides provide enough binding energy for measurable interaction with some SLE anti-ssDNA antibodies. The anti-ssDNA antibodies tend to have selectivity for particular bases, although in most cases, epitopes of anti-dsDNA are sugar-phosphate backbones of the helix (Stollar, 1994). There are also some reports showing a correlation between the occurrence of anti-RNA antibodies and SLE disease activity (Koffler et al., 1971; Eilat et al., 1978; Koffler et al., 1979; Stetler and Cavallo, 1987), although the co-relationship between them is not clearly established yet. Competitive ELISA showed that the antibodies produced in this report can be grouped into two different selectivities for DNA sequences; one type for A/T and the other for G/C. Although the antibodies show preferences for particular bases, they generally showed cross-reactivities to all other polynucleotides examined, suggesting that their epitopes are the overall shape or the outer features (sugar-phosphate backbones) of the helix, not base sequences. Some of them also have cross-reactivity with RNA. Markers recognizing common idiotypes such as cross-reactive anti-idiotype antibodies will be needed to identify shared features of the antibodies. Certain conformational features may be common to different primary structures and may be important for stimulation of idiotype networks that lead to the autoimmune disease (Shoenfeld and Mozes, 1990).

Many pathogenic antibodies are cationic antibodies, having a relatively high isoelectric point (Eilat, 1990; O'Keefe et al., 1992). The positive overall charge of cationic antibodies may facilitate their binding to negatively charged DNA, laminin, and components of the glomerular basement membrane (Sabbaga et al., 1989; O'Keefe et al., 1992; Termaat et al., 1992). The isoelectric points of the antibodies analyzed in this report were above 9.6, indicating that they are cationic antibodies.

The overall characteristics of the monoclonal autoantibodies suggest that pathogenic SLE antibodies are produced. The antibodies will be highly valuable for the chain dominance study and in the analysis of the primary structures of the antibodies. Future studies will enhance knowledge on the nature of the antigen binding sites and the interaction of antibodies to DNA.

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