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The production of antibodies to DNA in normal mice following immunization with poly(ADP-ribose)

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SUMMARY

Monoclonal antibodies were prepared from C57/black mice which had been immunized with poly(ADP-ribose). As expected, some of these antibodies were very specific for poly(ADP-ribose) but, surprisingly, several bound to DNA as well. Analysis of the sera of these mice also showed elevated levels of antibodies to DNA. Monoclonal antibodies against poly(ADP-ribose) were also recovered from unimmunized NZB/W mice which provided an animal model for systemic lupus erythematosus(SLE). These antibodies also cross-reacted with DNA. Comparison of the specificities of the monoclonal antibodies from the two groups of mice showed some striking similarities. In particular, three out of 11 antibodies from the C57/black mice preferred poly(dT) as judged by a solid phase radioimmunoassay. Similarly, 10 out of 17 antibodies from the NZB/W group showed the same type of specificity pattern. These results demonstrate that anti-DNA antibodies can be induced by poly(ADP-ribose) and that some of the autoimmune DNA-binding antibodies found in SLE may result from exposure to poly(ADP-ribose).

Keywords anti-poly(ADP-ribose) anti-DNA monoclonal antibodies NZB/W mice systemic lupus erythematosus

INTRODUCTION

Elevated levels of antibodies to an increasing number of nuclear antigens have been reported in sera of patients with systemic lupus erythematosus(SLE). Among these are antibodies to poly(ADP-ribose), a unique polymer synthesized from nicotinamide adenine dinucleotide(NAD) in cell nuclei (Kanai *et al.*, 1977; Sugimura *et al.*, 1980). While the precise functions of poly(ADP-ribose) are unknown, it appears to play a regulatory role in several cell processes including chromatin structure (Stone, Lorimer & Kidwell, 1977; Jump, Butt & Smulson, 1979), DNA repair (Nomura *et al.*, 1981; Benjamin & Gill, 1980), DNA synthesis (Burzio & Koide, 1970), cell differentiation (Caplan & Rosenberg, 1975; Ohashi *et al.*, 1984), and growth density (Stone & Shall, 1975).

Poly(ADP-ribose) may also play some role in the pathogenesis of SLE, since antibodies to this particular nuclear antigen have been reported to correlate more closely than anti-DNA antibodies with lupus disease activity (Okalie & Shall, 1979; Morrow *et al.*, 1982). In addition, there is evidence for the occurrence of circulating poly(ADP-ribose)-anti-poly(ADP-ribose) immune complexes in the sera of lupus patients (Okalie & Shall, 1979). Unlike DNA, poly(ADP-ribose) is immunogenic (Kanai *et al.*, 1974). Since DNA and poly(ADP-ribose) share some structural similarities, cross-reacting antibodies may in part account for the elevation of antibodies to each of these antigens seen in SLE.

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To test this hypothesis, we have prepared monoclonal antibodies from normal C57/black mice immunized with poly(ADP-ribose) and from unimmunized NZB/W female mice, an animal model for SLE. We have found that many of the monoclonal antibodies obtained bound not only to poly(ADP-ribose) but to several nucleic acids as well. In addition, binding patterns of the monoclonal antibodies obtained from each type of mouse were remarkably similar, suggesting that DNA need not necessarily be the immunogenic stimulus for the production of anti-DNA antibodies seen in SLE.

MATERIALS AND METHODS

Antigens. Poly(ADP-ribose) was produced by incubation of NAD with isolated calf thymus nuclei (Chauveau, Moule & Rouiller, 1956; Kanai *et al.*, 1980). The crude reaction mixture was treated extensively with pancreatic DNase and RNase as well as micrococcal nuclease. Final isolation of the poly(ADP-ribose) was achieved by hydroxyapatite chromatography as described previously (Sugimura *et al.*, 1971). Poly(ADP-ribose) purity was confirmed by spectral analysis (Minaga & Kun, 1983).

Double-stranded calf thymus DNA(nDNA) was purchased from Sigma. Heat-denatured calf thymus DNA(dDNA) was prepared by heating nDNA to 100°C for 10 min followed by quenching on ice. Poly(dT) was a product of P–L Biochemicals.

Immunization. Six 3-month-old female C57/black mice were injected intraperitoneally (i.p.) with 50 μg of poly(ADP-ribose) complexed to 50 μg of methylated bovine serum albumin(MBSA) in Freund's adjuvant on day 0. Intraperitoneal injections of 50 μg of poly(ADP-ribose) with 50 μg of MBSA in Freund's incomplete adjuvant followed on days 10 and 20. Six other 3-month-old female C57/black mice were injected in a similar fashion but with MBSA and adjuvant only (negative controls). All animals were bled and killed on day 23.

Monoclonal antibody production. Hybridoma cell lines were prepared as described previously (Lee *et al.*, 1981; Lee, Woodsworth & Latimer, 1984) from two of the poly(ADP-ribose) immunized mice and from seven unimmunized NZB/W female mice age 2–8 months (Jackson Laboratories, Bar Harbor, ME, USA). All hybridomas were cloned by limiting dilution. Supernatants from monoclonal cell lines were screened for anti-poly(ADP-ribose) activity by a solid phase radioimmunoassay(SPRIA).

SPRIA. SPRIA was performed as described previously (Lee *et al.*, 1981). Briefly, 96-well PVC plates were coated with 50 μl of 2 μg/ml of antigen in phosphate buffered saline(PBS) for at least 24 h. After three washes, 50 μl of either hybridoma supernatant or a 1/500 dilution of murine serum was added and incubated at room temperature for 2 h. After three more washes, 50 μl of I^{125} labelled goat anti-mouse antibody (Amersham, UK) was added and incubated a further 2 h. Finally, after three more washes, the individual wells were cut out and counted for 1 min in a gamma counter, with results expressed in counts per minute (ct/min). Total input per well of the labelled antibody was 20,000 ct/min. All washes were performing using PBS and 0·05% Tween 20. The sera of all mice were tested for antibody activity to poly(ADP-ribose), nCT, dCT, and poly(dT) and to a control well coated with PBS containing 1% fetal calf serum (FCS). Each sample was tested in duplicate. The same assay was used to test the specificity of the monoclonal antibodies. In this as in other studies (unpublished observations) a few monoclonal antibodies were encountered which bound to the plate even in the absence of antigen, i.e. a blank well. These antibodies are probably 'antiplastic' and were discarded.

Competition SPRIA on monoclonal antibodies was performed in an identical fashion except that the competing antigen was added immediately following the first set of washes (Lee *et al.*, 1984).

RESULTS

Previous studies have shown that poly(ADP-ribose) complexed to MBSA is immunogenic in both rabbits and mice (Kanai *et al.*, 1974; 1978; Kanai & Sugimura, 1981). As shown in Fig. 1, C57/black

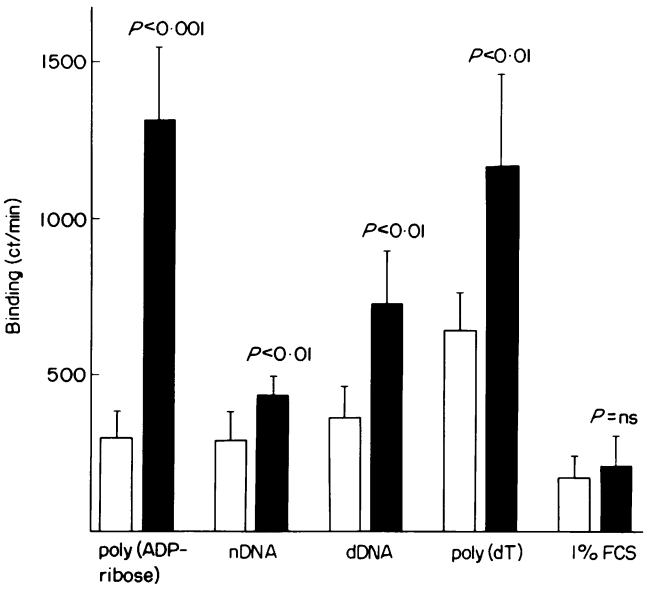

Fig. 1. Binding of serum antibodies (1/500 dilutions) to various antigens measured by the SPRIA. (■) C57/black mice immunized with poly(ADP-ribose) complexed to MBSA. (□) Control mice injected with MBSA alone. The results are shown for the average (±standard deviations) for six mice in each group. The *P* values (immunized *v* control mice) were significant for the nucleic acid antigens but not for wells coated with 1% fetal calf serum (1% FCS).

mice produce a good immune response to poly(ADP-ribose) after a series of injections of the antigen-MBSA complex with accompanying adjuvant. Surprisingly however, antibodies are also found which bind to nDNA, dDNA and poly(dT) (Fig. 1). The level of antibody to each of these antigens is significantly greater than that seen following serial injections of MBSA in Freund's adjuvant without poly(ADP-ribose). In order to investigate the specificity of this response, monoclonal antibodies were produced.

Hybridomas were produced from the spleen cells of two C57/black female mice immunized with poly(ADP-ribose) as described above. Hybridoma supernatants were screened with SPRIA for the production of antibodies which bound to poly(ADP-ribose). Despite this selection, eight of the eleven hybridomas obtained also showed high levels of binding to nDNA, dDNA and poly(dT) (Table 1). Indeed for three of the antibodies (ADP–2, ADP–3 and ADP–5), it would appear that poly(dT) is the preferred antigen. Thus immunization with poly(ADP-ribose) produces antibodies that cross-react with DNA. As expected, some of the antibodies (ADP–1, ADP–6, and ADP–9) seem quite specific for poly(ADP-ribose) itself.

Similarly, 17 hybridomas that produced poly(ADP-ribose)-binding antibodies were obtained from the spleen cells of seven unimmunized NZB/W female mice aged 2–8 months. SPRIA results for the binding of these antibodies to poly(ADP-ribose), nDNA, dDNA and and poly(dT) are shown in Table 2. In this case, none of the antibodies showed a marked preference for poly(ADP-ribose). However, for 10 out of this group of 17, poly(dT) was again the preferred antigen. Thus the specificity patterns of some of the antibodies from both groups of mice are quite similar.

Although the SPRIA gives a useful indication of specificity, the results may be misleading since an antibody may appear to bind well to two antigens even though their binding constants differ by over 100-fold (Lee, Dombroski & Mosmann, 1982). For this reason, competition SPRIA was also performed to elucidate the relative binding to various antigens. It was found that four monoclonal antibodies from the immunized C57/black mice and four antibodies from the unimmunized NZB/W mice were competed well with at least one antigen. This is shown in Figs 2 and 3 respectively.

As expected from the SPRIA results, ADP–1 is competed only by poly(ADP-ribose). However,

Table 1. SPRIA results (%maximum)* for monoclonal antibodies produced from C57/black mice immunized with poly(ADP-ribose)

Antibody ADP no.	poly(ADP-ribose)	Antigens†			
		nCT	dCT	poly(dT)	1% FCS
1	100	<5	<5	<5	<5
2	29	10	49	100	<5
3	68	65	53	100	<5
4	87	100	92	98	20
5	63	62	77	100	<5
6	100	10	<5	<5	<5
7	100	43	36	61	18
8	100	27	37	56	17
9	100	7	<5	8	<5
10	100	42	32	27	<5
11	100	33	24	32	<5

* Results are expressed as a percentage of maximum binding after subtraction of the background. The maximum ct/min were approximately 2,000 with a background ct/min of less than 200.

† The wells were coated with the nucleic acid antigens at 2 μg/ml. 1% FCS signifies wells coated with a solution of 1% fetal calf serum.

Table 2. SPRIA results (% maximum) *from autoimmune NZB/W mice

antibody NZ no.	poly(ADP-ribose)	Antigens†			
		nCT	dCT	poly(dT)	1% FCS
1	96	63	73	100	<5
2	95	97	96	100	<5
3	57	46	48	100	<5
4	100	95	91	94	18
5	51	45	46	100	<5
6	95	88	86	100	8
7	100	57	34	25	<5
8	34	73	76	100	<5
9	43	27	62	100	<5
10	61	34	20	100	11
11	62	43	44	100	10
12	38	79	100	91	<5
13	15	96	100	84	<5
14	39	43	100	79	12
15	88	73	100	67	19
16	51	50	60	100	9
17	74	67	100	83	11

* Results are expressed as a percentage of maximum binding after subtraction of the background. The maximum ct/min were approximately 3000 with a background ct/min of less than 200.

† The wells were coated with the nucleic acid antigens at 2 μg/ml. 1% FCS signifies wells coated with a solution of 1% fetal calf serum.

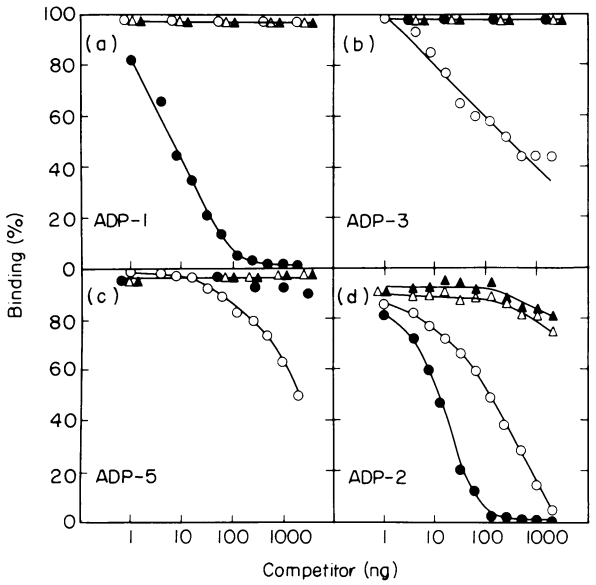

Fig. 2. Competition binding experiments with monoclonal antibodies from C57/black mice immunized with poly(ADP-ribose). The per cent inhibition of binding to poly(ADP-ribose) is shown as a function of the amount of nucleic acid added as competitor in the SPRIA. (●) poly(ADP-ribose); (○) poly(dT); (△) nDNA, (▲) dDNA.

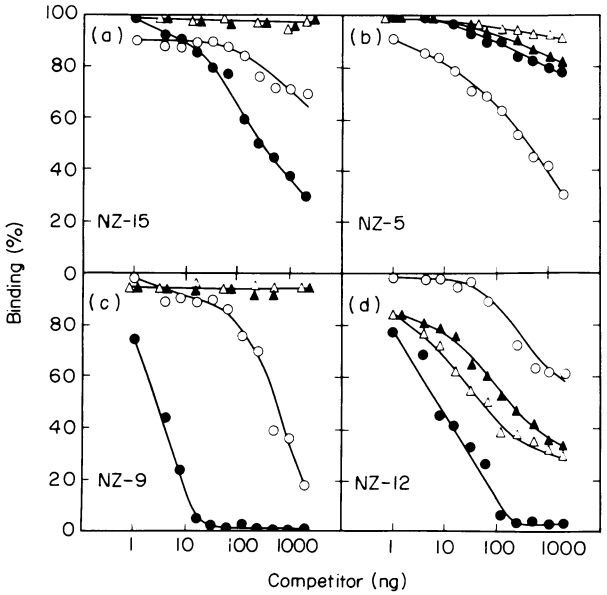

Fig. 3. Competition binding experiments with monoclonal antibodies from autoimmune NZB/W mice. The per cent inhibition of binding to poly(ADP-ribose) is shown as a function of the amount of nucleic acid added as competitor in the SPRIA. (●) poly(ADP-ribose); (○) poly(dT); (△) nDNA; (▲) dDNA.

somewhat surprisingly, ADP–3 and ADP–5 are competed only by poly(dT), even though the SPRIA suggested some binding to the other nucleic acid antigens as well. Since poly (ADP-ribose) is required on the plate for binding to occur (i.e. these are not 'antiplastic' antibodies) then presumably, the binding constant to poly(dT) is considerably larger than to poly(ADP-ribose). Or, in other words, unrealistically high concentrations of poly(ADP-ribose) would have to have been

used in order to observe competition. This experiment, therefore, confirms that a DNA-binding antibody can be induced by immunization with poly(ADP-ribose). On the other hand, ADP–2 is competed by both poly(dT) and poly(ADP-ribose), although poly(ADP-ribose) is the preferred antigen.

In the case of the monoclonal antibodies from the NZB/W mice, three of them show strikingly similar specificity patterns to those derived from the mice immunized with poly(ADP-ribose). That is, NZ–15 and ADP–1 are competed only by poly(ADP-ribose), NZ–5, ADP–3 and ADP–5 by poly(dT) only, and NZ–9 and ADP–2 by both poly(ADP-ribose) and poly(dT). Thus many of the spontaneous NZB/W autoantibodies appear to be similar to those derived from the C57/black mice immunized with poly(ADP-ribose). NZ–12, on the other hand, is competed not only by poly(ADP-ribose) but also by dCT, nCT and to a much lesser extent poly(dT).

DISCUSSION

It has previously been shown that immunization with cardiolipin can produce anti-DNA antibodies in both rabbits and mice (Guarnieri & Eisner, 1974; Rauch *et al.*, 1984). It has been suggested that the phosphate-sugar-phosphate moeity of cardiolipin mimics the backbone of DNA, thus explaining the cross-reactivity of antibodies to both antigens (Rauch *et al.*, 1984).

In the present study we have demonstrated that immunization with poly(ADP-ribose) can also induce antibodies to DNA, particularly to the synthetic nucleic acid, poly(dT). However, in this case, the repeating backbone structure of poly(ADP-ribose) (i.e. ribose-ribose-phosphate-phosphate) is rather different from that of DNA so that a simple explanation for the observed cross-reactivity is difficult to derive. For example, ADP–2, as judged from competition experiments, binds to both poly(ADP-ribose) and poly(dT), which might suggest that the common epitope is (ribose-phosphate). However, dCT contains (ribose-phosphate) groups in abundance and yet competition with dCT is barely detectable. Thus the most plausible explanation for this puzzling specificity pattern seems to be that the different antigens have different (but possibly overlapping) binding sites on the antibody.

On the other hand some of the monoclonal antibodies (e.g. ADP–1) from the immunized mice are very specific for poly(ADP-ribose) and do not bind DNA. Similar highly specific monoclonal antibodies to poly(ADP-ribose) were recently reported by Kawamitsu *et al.* (1984) who elucidated some of the functional groups being recognized.

Kanai *et al.* (1985) have reported that MRL/1 mice, an animal model for SLE, can spontaneously produce antibodies to poly(ADP-ribose) that cross-react with both single-stranded DNA and left-handed Z DNA. Using another animal model for SLE, we have shown that NZB/W female mice also spontaneously produce antibodies to poly(ADP-ribose) that cross-react with DNA. In addition, those monoclonal antibodies are remarkably similar in specificity to monoclonal antibodies derived from C57/black mice immunized with poly(ADP-ribose). It has been found in this study and also from previous work (Lee *et al.*, 1982) that many autoimmune antibodies bind well, if not best, to poly(dT). It is thus intriguing that poly(ADP-ribose) can induce the production of antibodies to poly(dT).

It has recently been shown that autoimmune mice show a normal immune response to antigens other than DNA (Madaio *et al.*, 1984), suggesting that the immune defect is a rather selective one. Is it possible that this defect involves loss of tolerance to such antigens as poly(ADP-ribose)? The production of antibodies to DNA would be envisaged, therefore, as a consequence of an immune response to poly(ADP-ribose).

Photosensitivity is a common feature in SLE. Indeed, flare of disease activity in some patients following exposure to ultraviolet light, is a well recognized phenomenon. Excessive exposure to ultraviolet light would be expected to produce DNA damage, which in turn is known to lead to an increased synthesis of poly(ADP-ribose) (Nduka, Skidmore & Shall, 1980). If this antigen escapes from damaged cells then an immune response to both poly(ADP-ribose) and DNA might be generated. Although highly speculative, the possibility is raised that autoimmune diseases such as SLE may in part be caused by a defect in metabolism of potential antigens such as poly(ADP-ribose), rather than exclusively by a defect in the immune system itself.

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