Clin. exp. Immunol. (1984) **58,** 293–299.

Induction of anti-DNA autoanti-idiotypic antibodies in (NZB × NZW)F_1 mice: possible role for specific immune suppression

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(Accepted for publication 19 June 1984)

SUMMARY

(NZB × NZW)F_1 (B/W) mice spontaneously produce anti-deoxyribonucleic (DNA) acid antibodies. PME77 anti-DNA monoclonal antibody (MoAb) is a syngeneic antibody bearing idiotype present in most B/W sera. In the present investigation the effect of immunization of B/W mice with the PME77 MoAb on the production of PME77 idiotypes and anti-DNA antibodies in B/W mouse sera was investigated. PME77 MoAb immunization regimen induced the production of autoanti-idiotypic antibodies and abrogated the expression of PME77 idiotype in B/W treated mice. In contrast, untreated mice and control B/W mice, receiving NZB polyclonal IgG2b which lacked detectable DNA binding capacity, expressed PME77 idiotopes. These results demonstrate that the expression of idiotype borne by autoantibodies may be modified through the induction of autoanti-idiotypic antibodies.

Keywords lupus erythematosus monoclonal antibodies anti-DNA antibodies autoanti-idiotype idiotypic suppression

INTRODUCTION

(NZB × NZW)F_1 hybrid mice (B/W) develop a spontaneous disease resembling human systemic lupus erythematosus (SLE). These mice produce a variety of anti-nucleic acid autoantibodies, including antibodies directed against deoxyribonucleic acid (DNA), which are thought to play a major role in the pathogenesis of the disease (Dixon *et al.*, 1980).

Immune manipulations, using non antigen specific immunosuppressive therapies, have been proposed for suppressing the production of anti-nucleic acid antibodies in SLE mice (review in Tron, Jacob & Bach, 1983). Selective suppression of antibodies to nucleic acid antigens might constitute a new therapeutic of SLE. To achieve this selectivity, two approaches may be attempted. First, treatment of B/W mice with nucleoside coupled to isogeneic spleen cells was shown to elicit nucleoside specific suppressor T cells (Borel & Young, 1980). Second, another approach would be to use antibodies to manipulate the immune response through the idiotype–anti-idiotype network (Jerne, 1974).

Idiotypes are antigenic determinants found on antibody of a particular specificity (Oudin & Michel, 1963). Studies of inbred mice demonstrated that certain idiotype are present in every individual of a particular strain immunised with a given antigen (recurrent idiotype). Anti-idiotypic antisera directed to recurrent idiotypes may induce a suppression of antibody bearing these idiotypes (review in Mäkelä & Kajalainen, 1977).

Moreover, experiments suggested that immunization by the idiotype itself may suppress the antibodies with the idiotype (Rowley *et al.*, 1976) through the development of suppressor cells (Eichman *et al.*, 1978).

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The production of anti-DNA monoclonal antibodies (MoAb) by the hybridoma technology from non-immunized autoimmune mice have showed that anti-DNA MoAb share similar idiotypic determinants (Rauch *et al.*, 1982; Marion *et al.*, 1982). We also reported the production of anti-DNA MoAb whose antigenic specificities were demonstrated to be identical and directed against the B helical form of DNA (Tron *et al.*, 1980; Jacob & Tron, 1982). One of these, PME77 MoAb, was shown to bear idiotypic determinants present in most B/W sera (Tron *et al.*, 1983).

In the present investigation, the effect of immunisation of B/W mice with PME77 MoAb on the production of PME77 idiotypes and anti-DNA antibodies in B/W mice sera was investigated.

MATERIALS AND METHODS

Mice. NZB, BALB/c, C57BL/6 mice were obtained from the CSEAL-CNRS (Orléans, La Source, France). NZW mice were kindly provided by Dr Verroust. B/W mice were the offspring of NZB females and NZW males and were 3 months old at the onset of experiments.

Nucleic acid. DNA (calf thymus) and ribonucleic acid (RNA) were purchased from Worthington (Biochemical Corp., Freehold, New Jersey, USA).

DNA binding capacity. DNA binding capacities of B/W mouse sera were measured using a cellulose ester filter radioimmunoassay, with ^{14}C-DNA from *E. Coli* (Amersham, Le Vésinet, France) (Attias, Sylvester & Talal, 1973).

Cell fusion. The hybridoma secreting anti-DNA antibodies were obtained following fusions between a non secreting myeloma line (P3 × 63-Ag 8653) and B/W spleen cells. The selection of hybrid producing PME77 anti-DNA antibody, cloning and subcloning of the line were all described in detail previously. PME77 MoAb was shown to be an IgG2b,κ chain antibody (Tron *et al.*, 1980).

Purification of anti-DNA MoAb. Culture media were collected from the established hybridoma cell lines. Immunoglobins (Ig) were precipitated in 50% saturated ammonium sulphate (SAS) and dialysed in 0·15 M phosphate-buffered saline (PBS). The MoAb were purified by affinity chromatography as previously described (Tron *et al.*, 1980).

Preparation of rabbit anti-idiotype antiserum. An anti-idiotype xeno-antiserum against PME77 MoAb was prepared by immunizing rabbits as described previously (Tron *et al.*, 1983). This anti-idiotype antiserum was shown to detect ligand modifiable idiotypic determinants on the PME77 molecule. These idiotypic determinants were demonstrated to be present at low levels in most B/W sera tested (Tron *et al.*, 1983).

Radioiodination. Thirty micrograms of purified PME77 MoAb were radioiodinated according to Hunter's protocol (Hunter, 1970) (specific activity: 5×10^4 ct/min/μg).

Experimental protocol. The basic experimental design was as follows. A suspension of 100 μg of PME77 anti-DNA antibody co-polymerized with keyhole limped haemocyanin (Calbiochem., San Diego, California, USA) (KLH/PME77 ratio: 1/1) by means of glutaraldehyde was given subcutaneously to a group of 10 B/W female mice starting at 3 months of age (group A). The suspension was mixed with an equal volume of Freund's complete adjuvant (DIFCO, Detroit, Michigan, USA) and was injected into footpads. The second immunisation was given in Freund's incomplete adjuvant (DIFCO). Booster immunizations were in saline and repeated monthly. Treatment ended on day 210. Control groups consisted of unimmunized mice (group C) and of mice receiving polyclonal NZB IgG2b (group B) that lacked detectable anti-DNA activity, according to the protocol described above (six mice). On days 60, 120, 150, 180 and 210 after the first immunization, sera were assayed for DNA binding capacities, idiotype and anti-PME77 idiotype contents.

Solid phase radioimmunoassay (RIA). The binding of PME77 mAb to rabbit anti-PME77 idiotype was studied in the solid phase RIA as described by Tron *et al.* (1982). Briefly disposable flexible polyvinyl chloride microtitre plates were coated with rabbit anti-PME77 idiotype IgG fraction. The free binding sites on the plastic surface was saturated with 0·5% bovine serum albumin (BSA) in PBS. Fifteen thousand ct/min of the ^{125}I-labelled PME77 MoAb was added. The inhibition of binding of ^{125}I-labelled PME77 MoAb to its homologous anti-idiotype was performed by

incubating various dilutions of sera obtained from the three groups of B/W mice. After washing the plates were cut into individual well and the bound radioactivity determined.

Characterization of the material giving an inhibition in the solid phase radioimmunoassay. Sera collected from the same group of B/W mice at the same period were pooled. Immunoglobulins were isolated on an affinity column: the Ig fraction of a goat anti-mouse Ig was attached to pre-activated Sepharose 4B (S4B) (Pharmacia, Pistacaway, New Jersey, USA) according to the manufacturer's instructions. The column was loaded with B/W mouse sera and washed with PBS. The elution were performed using 0·1 M glycine, HCl pH 2·3. The eluted material was buffered to neutrality with Tris-HCl pH 9.

Purified Ig were tested in a solid phase radioimmunoassay. First, purified Ig were coated on plastic plates as described above. ^{125}I-rabbit anti-PME77 idiotype or ^{125}I-PME77 were used as tracers. Second PME77 was coated to plastic plates and B/W purified IgG were used as tracers.

RESULTS

To determine whether the anti-DNA antibody production can be modified by immunising B/W mice with PME77 MoAb, 10 mice were treated with PME77 MoAb. Five B/W mice were similarly immunized with (non-anti-DNA) polyclonal IgG2b and 10 mice were non-immunized.

At the beginning of the immunization program all B/W mice of the three groups had undetectable PME77 idiotypes as assessed in the PME77/anti-idiotype PME77 and normal DNA binding capacities.

On day 60, a strong inhibition of the PME77/anti-PME77 reaction was given by five out of 10 sera of the PME77 treated mice. This inhibition was observed in all mice of this group from day 120 to day 210. However, the titre of sera giving 50% inhibition, which was as high as 1/1280 in certain animals decreased with time in most mice (Table 1). In IgG2b treated control mice and in untreated mice a weak inhibition of the PME77/anti-PME77 reaction was also observed (Table 2).

Characterization of the material giving an inhibition in the PME77/anti-idiotype reaction in PME77 treated and untreated mouse sera

Ig from PME77 treated mice were pooled and purified by affinity chromatography. Purified Ig gave a strong inhibition of the PME77/anti-PME77 reaction (Fig. 1). Experiments summarized in Table 3 showed that these Ig contained autoanti-idiotypic antibodies and that no PME77 idiotype could be detected. First, when coated to plastic plates, these Ig bound ^{125}I-PME77 MoAb but not ^{125}I-anti-PME77 anti-Id xenoantiserum. Second, when ^{125}I-labelled, these Ig bound PME77 MoAb

Table 1. Inhibition of binding of ^{125}I-PME77 to PME77 anti-Id Xenoantiserum by 10 sera from mice treated with PME77 anti-DNA MoAb (group A)

		Unlabelled inhibitor									
Day after the first immunization	PME77 MoAb*	B/W No.† 1	2	3	4	5	6	7	8	9	10
0	25	—‡	—	—	—	—	—	—	—	—	—
60	25	—	—	—	—	640	640	1,280	—	640	1,280
120	25	320	160	320	320	160	320		320	320	640
150	25		80	80	80	80	160		80	80	320
180	25		80	160	80	160	320		80	160	160
210	25		80	80	80	160			160	80	80

* Results are expressed as nanograms required for 50% inhibition.

† Results are expressed as reciprocal dilution required for 50% inhibition.

‡ A weak inhibition is observed.

coated to plastic plates. In contrast, untreated or treated control mouse Ig coated to plastic plates bound to ^{125}I-rabbit anti-PME77 idiotype but not to ^{125}I-PME77 MoAb which demonstrate that the inhibition is given by the presence of PME77 idiotype.

Characterization of the autoanti-idiotypic antibodies induced in treated B/W mice

Inhibition of binding of PME77 MoAb to its homologous autoanti-idiotype antibodies was studied in the solid phase RIA described in Materials and Methods. The reaction was inhibited by bacteriophage dsDNA but not by ribonucleic acid (Fig. 2). Table 4 shows that PME77 MoAb and rabbit anti-PME77 antiserum are potent inhibitors of the reaction. In contrast, none of the other IgG2a and IgG2b anti-DNA MoAb studied gave an inhibition of the binding of PME77 MoAb to the autoanti-idiotype antibodies.

Effect of the induction of autoanti-PME77 idiotypic antibodies on the DNA binding capacities in B/W mouse sera

The induction of anti-PME77 idiotype antibodies and the suppression of PME77 idiotypes did not change the total DNA binding capacities of the sera of treated B/W mice, compared to control and untreated B/W mice.

Table 2. Inhibition of binding of ^{125}I-labelled PME77 to it's rabbit anti-Id antiserum by five sera from B/W mice treated with immunoglobulin (group B) and by 10 sera from B/W mice non-immunized (group C)

	Unlabelled inhibitor Mouse No.	ng of idiotype present in B/W mouse sera at the dilution of 1/20
Group B	1	2·5
	2	10
	3	2·5
	4	—
	5	—
Group C	1	—
	2	5
	3	5
	4	—
	5	—
	6	5
	7	—
	8	10
	9	5
	10	2·5

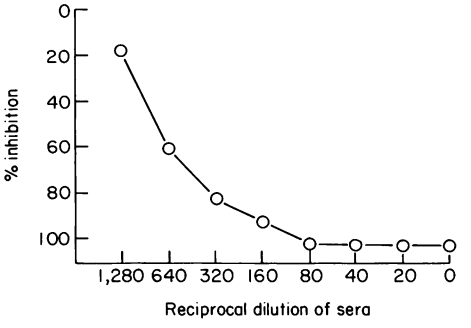

Fig. 1. Inhibition of binding of ^{125}I-labelled PME77 to its rabbit anti-Id antiserum by purified Ig from 10 B/W mice sera immunized against PME77 MoAb.

Table 3. Binding experiment using PME77 MoAb, rabbit anti-PME77 and purified mouse Ig as tracers or plastic plate coated proteins

^{125}I-tracers† Coated to plastic plate	PME77 MoAb	Rabbit anti-PME77	Treated mouse Ig	Control Ig	Untreated mouse Ig
PME77*	ND	2,800±173	812±20	88±22	136±19
Rabbit anti-PME77	4,815±230	ND	120±26	620±82	542±124
Treated mouse Ig	1,024±60	ND	ND	ND	ND
Control mouse Ig	87	248	ND	ND	ND
Untreated mouse Ig	75	213	ND	ND	ND

* PME77 MoAb was coated at the concentration of 5 μg/ml; rabbit anti-PME77 was coated at the concentration of 20 μg/ml; other reagents were coated at the concentration of 100 μg/ml.

† Specific activity of the ^{125}I-tracers ranged from 5×10^4 to 10^5.

Results are given in ct/min bound per plate.

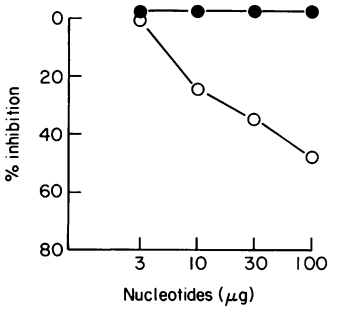

Fig. 2. Inhibition of binding of ^{125}I-labelled B/W anti-Id IgG to PME77 antibody by bacteriophage lambda (dsDNA, ○) and by double stranded (dsRNA, ●).

Table 4. Idiotypic specificity of B/W antiserum directed against PME77 MoAb

Unlabelled inhibitor		No. of ct/min bound per plate	% inhibition
Background		75±15	
None		920±56	
NMS+	10	898±37	<10%
	20	943±53	<10%
	40	924±65	<10%
PME77 MoAb (ng)	300	82±13	100
	100	213±27	76
	30	481±51	48
	10	724±63	24
	3	936±72	20
Rabbit anti-PME77 antiserum (ng)	300	78±16	100
	100	341±47	63
	30	626±73	32
	10	907±67	20

NMS+ = normal mouse sera (reciprocal dilution).

DISCUSSION

To suppress the PME77 idiotype production in 10 B/W mice, these animals were repeatedly immunized against the PME77 anti-DNA MoAb, starting at 3 months of age. PME77 idiotopes were not detectable in treated B/W mouse sera as well as in control mouse sera before immunization. PME77 immunization regimen induced the production of autoanti-idiotypic antibodies and abrogated the expression of PME77 idiotopes in B/W treated mice. In contrast, untreated B/W mice or control B/W mice expressed PME77 idiotopes. These results demonstrate that the expression of idiotype borne by autoantibodies may be modified through the induction of autoanti-idiotypic antibodies. Whether the suppression of PME77 idiotype in treated B/W mouse sera is achieved through cellular mechanisms or the formation of autoanti-idiotype/idiotypes complexes remains to be determined.

No decrease of the DNA binding capacities of B/W mice treated was observed. This is not surprising since it was previously shown that anti-DNA antibodies bearing PME77 idiotypes are a minor part of the whole anti-DNA antibody population (Tron *et al.*, 1983).

In contrast, Hahn & Ebling (1983) reported the suppression of part of circulating anti-DNA antibodies by repeated administrations of an IgG2a anti-DNA MoAb to B/W mice. The glomerular deposition of IgG and cationic IgG anti-DNA antibodies thought to be characteristic of murine lupus nephritis were diminished. The role of anti-idiotypic antibodies was suggested. Moreover, there is some evidence that the presence of anti-idiotypic antibodies directed against anti-DNA antibodies in SLE patient serum is responsible for clinical remissions (Abdou *et al.*, 1981).

Autoanti-idiotypic antibodies have been shown to be efficient in preventing experimentally induced renal disease. Nelson & Philipps (1982) induced antibody-mediated interstitial nephritis by immunizing rats with renal tubular antigens. These animals were protected from the development of nephritis by induction of autoanti-idiotypic antibodies against idiotypes present within the antigen binding region of anti-tubular basement membrane MoAb.

Taken together these results suggest that by immunizing B/W mice with MoAb bearing major recurrent idiotopes the production of anti-DNA antibodies in B/W mice sera might be suppressed. Finally, our results open the possibility of using autoanti-idiotypic reagents as specific suppressors of recurrent anti-DNA idiotypes in adult B/W mice. This approach will permit the study of the cellular events that precede the production of anti-DNA antibodies. In the future, it will also constitute the first step of a new therapeutic antigen specific approach of autoimmune diseases.

We wish to thank Marie-Annick Lety and Béatrice Legrand for their excellent technical assistance.

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