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Suppression of anti-DNA antibody production in MRL mice by treatment with anti-idiotypic antibodies

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SUMMARY

Antibodies against idiotypic determinants carried by a monoclonal polyspecific natural autoantibody were raised in rabbits and in syngeneic BALB/c mice. These anti-idiotypic antibodies were administered to newborn and to pregnant BALB/c mice and to MRL-1pr/1pr mice. Serial measurements of the idiotypes, naturally occurring autoantibodies, and antibodies obtained after antigenic stimulation were performed in the sera of the injected mice and in the offspring of pregnant mice. No idiotypic suppression was noted in newborn injected mice. Transient suppression of idiotypes recognized by the syngeneic anti-idiotypic antibody was noted in the offspring of pregnant mice injected with the rabbit polyclonal anti-idiotypic antiserum. No changes in naturally occurring autoantibodies or in antibodies appearing after antigenic stimulation were noted in BALB/c mice. In contrast, a significant decrease of spontaneously occurring anti-DNA antibodies was found in MRL-1pr/1pr mice treated with rabbit polyclonal anti-idiotypic antiserum. Furthermore in these mice a slight decrease of anti-TNP antibodies was also observed. These results suggest that anti-idiotypic antibodies directed against natural autoantibodies may play a regulatory role in the immune system; this role is more easily appreciated in mice suffering from immune dysregulation.

Keywords natural autoantibodies polyspecificity public idiotypes

INTRODUCTION

A considerable number of studies have been performed on idiotypic regulation of the immune system since the initial formulation of the idiotypic network hypothesis (Jerne, 1974). Accumulated evidence clearly indicates that the interaction of idiotopes with anti-idiotypic antibodies results in immune modulation characterized either by suppression or by enhancement of these idiotopes (Eichmann, 1974; Accolla *et al.*, 1977; Kim & Hopkins, 1978; Fung & Köhler, 1980a, b).

Investigators have also tried to assess the importance of the idiotypic network in autoimmune disease. Anti-idiotypic antibodies have been shown to decrease the production of rat anti-thyroglobulin antibodies and to induce tolerance (Zanetti & Bigazzi, 1981). In other studies with NZB mice, idiotypes were injected and the appearance of autoanti-idiotypic antibodies was examined yielding conflicting results (Hahn & Ebling, 1983; Jacob & Tron, 1984). Spontaneous generation of autoanti-idiotypic antibodies has also been studied during remission in myasthenia gravis (Dwyer *et al.*, 1983).

Studies in our laboratory have demonstrated that natural polyspecific autoantibodies exist in the sera of various animal species (Karsenti *et al.*, 1977a, b; Avrameas, Guilbert & Dighiero, 1981;

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Guilbert, Dighiero & Avrameas, 1982; Dighiero, Guilbert & Avrameas, 1982). Further studies with hybridomas from normal or immunized mice have confirmed these observations (Dighiero *et al.*, 1983; 1985; Guilbert *et al.*, 1985). More recently, we prepared polyclonal rabbit and murine antibodies directed against idiotypic determinants of monoclonal natural polyspecific autoantibodies (Lymberi *et al.*, 1985). Rabbit polyclonal anti-idiotypic antibodies were generated that recognize recurrent idiotopes present in most polyspecific natural autoantibodies. Syngeneic anti-idiotypic antisera recognize private idiotopes (Lymberi *et al.*, 1985 and unpublished results). Finally, it has been shown that monoclonal polyspecific autoantibodies derived from 6-day-old mice could participate in idiotypic/anti-idiotypic interactions (Holmberg *et al.*, 1984).

These results led us to study the possible biological effects of rabbit and syngeneic mice antibodies raised against the idiotypic determinants of an IgM monoclonal polyspecific autoantibody: D23. This naturally occurring autoantibody recognizes primarily ds and ssDNA.

Anti-idiotypic antibodies were administered to newborn and to pregnant BALB/c mice. Furthermore, because we found idiotypic determinants of the D23 autoantibody in the sera of MRL-lpr/lpr autoimmune mice with a lupus-like syndrome associated with high titres of anti-DNA antibodies (Murphy & Roths, 1978), we tested also the effect of anti-idiotypic antibodies in these mice.

The results obtained suggest that anti-idiotypic antibodies to natural autoantibodies may alter the immune response in autoimmune MRL-lpr/lpr mice.

MATERIALS AND METHODS

Animals. Newborn and adult BALB/c and MRL-lpr/lpr mice, purchased from the Jackson Laboratory, Bar Harbor, Maine, USA, were maintained in the animal colony of the Pasteur Institute.

Reagents. Gelatin was purchased from Prolabo (Paris, France). Tween 20 was purchased from E. Merck (Darmstadt, FRG). 'Luxlon' 96 well flat-bottomed microtitration plates were obtained from CML (Nemours, France). O-nitrophenyl-β-D-galactopyranoside (ONPG) and O-phenylenediamine (OPD) were purchased from the Sigma Chemical Co. (St Louis, MO, USA). *Escherichia coli* β-galactosidase (Sp. act. 500,000 U/ng) was kindly supplied by Dr A. Ullmann, Institut Pasteur (Paris, France).

Antigen sources. Actin was purified from striated muscle of BALB/c mice as described by Spudish & Watt (1971). Myosin was prepared from the same source as described by Whalen, Butler-Browne & Gros (1978). Mice brain tubulin was prepared according to the method of Shelansky, Gaskin & Cantor (1973). Human albumin was purchased from Shwartz Mann (Cambridge, USA). Mice transferrin was purchased from Cappel Laboratories (Cochranville, PA, USA). Purified human transferrin was obtained from Behringwerker AG (Marburg, FRG). Calf thymus native double-stranded DNA, trinitrobenzene sulphonic acid (TNBS) and keyhole limpet haemocyanin (KLH) were purchased from the Sigma Chemical Co. (St Louis, MO, USA). TNP was coupled to bovine serum albumin (BSA) according to the method of Little & Eisen (1966); 25 groups of TNP were coupled per molecule of BSA (TNP_{25}/BSA). Methylated BSA-DNA complexes were prepared as described by Plescia, Braun & Palczuk (1964).

Natural monoclonal antibody. The monoclonal D23 antibody (IgM.k) was obtained by fusing the splenocytes of 12-week-old unprimed BALB/c mice with the non-secreting myeloma cell line SP_2/O (Dighiero *et al.*, 1983). This antibody reacts mainly with dsDNA and ssDNA, but also with actin, tubulin, myosin, α-fetoprotein, myoglobin, transferrin, thyroglobulin, spectrin, and TNP/BSA (Lymberi *et al.*, 1985).

Preparation of anti-idiotypic antibodies (anti-IdD23). Rabbit antibodies directed against the idiotypic determinants of D23 (IdD23) were obtained as described in detail elsewhere (Lymberi *et al.*, 1985). The specificity of these antibodies was assessed by ELISA (Lymberi *et al.*, 1985). Syngeneic mice antibodies directed against idiotopes of D23 were obtained by immunizing BALB/c mice with conjugates of D23 with KLH prepared using *p*-benzoquinone as the coupling agent (Ternynck & Avrameas, 1976). Conjugates (100 μg) in complete Freund's adjuvant were injected

Table 1. Treatment of mice with anti-idiotypic (IdD23) antibodies

Strain	*n*	Quantity of antibody (μg)	Age at injection
Rabbit anti-IdD23*			
BALB/c	20	10	1 day
	20	50	1 day
	20	200	1 day
Pregnant BALB/c mice	3	500	3 months (days 16–19 of pregnancy)
MRL-lpr/lpr	20	50	1 day

* Purified rabbit IgG anti-IdD23 was injected intraperitoneally. Control mice received normal rabbit IgG.

intradermally and intramuscularly four times at 30-day intervals. The presence of anti-idiotypic antibodies was assessed by the Ouchterlony technique. The most positive sera were then selected, mixed and run through a protein A-Sepharose column in order to isolate the mouse IgG (Ey, Prowse & Jenkin 1978). The specificity of this IgG antibody was assessed by ELISA.

Treatment of mice with anti-idiotypic antibodies. Group of mice were injected intraperitoneally (i.p.) with a single dose of rabbit anti-IdD23 as described in Table 1. Newborn BALB/c mice were treated with syngeneic anti-IdD23 at days 1, 3 and 5 with respectively 50 μl, 50 μl and 100 μl of whole anti-serum. Control mice received either rabbit IgG isolated from a normal serum pool or normal BALB/c serum.

Bleeding and immunization of mice. BALB/c mice were bled at 1 and 2 months of age, and then immunized (i.p.) with either 50 μg of TNP_{25}/BSA or 100 μg of myosin, transferrin, or methylated BSA-DNA complex in complete Freund's adjuvant. Animals were bled 20 days after antigen injection.

MRL-lpr/lpr mice were bled at 1 month of age and every 15 days thereafter for a period of 4 months.

All the sera were tested by ELISA as described below.

ELISA for the titration of antibodies. Serial dilutions of the mouse sera were incubated in plates coated with the various antigens used for immunization. After 1 h at 37°C, the plates were washed, incubated with a β-galactosidase-labelled sheep anti-mouse Ig antibody, re-washed, and incubated with substrate before measurement of enzyme activity. The detailed procedure has been described elsewhere (Guilbert, Dighiero & Avrameas, 1982).

ELISA for the quantification of IdD23. IdD23 in the various mouse sera was measured using rabbit IgG anti-IdD23 antibody as described previously (Lymberi *et al.*, 1985).

To measure IdD23 using the mouse syngeneic anti-IdD23 antibody, plates coated with mouse IgG antibody were used. Mouse sera were incubated in these plates, and after washing, the same β-galactosidase-labelled mouse antibody was added. The ELISA was completed as described above.

RESULTS

Anti-idiotypes and idiotopes. The characteristics of the rabbit anti-IdD23 antibody have been described previously (Lymberi *et al.*, 1985). This antibody recognizes recurrent idiotopes present in most natural monoclonal polyspecific autoantibodies. Using plates coated with this anti-idiotypic antibody, we looked for IdD23 determinants in the sera of MRL-lpr/lpr and BALB/c mice (Fig. 1). The sera of MRL mice and BALB/c mice contain comparable amounts of IdD23 determinants whether the test is performed using a rabbit anti-mouse Ig or a rabbit anti-mouse IgM.

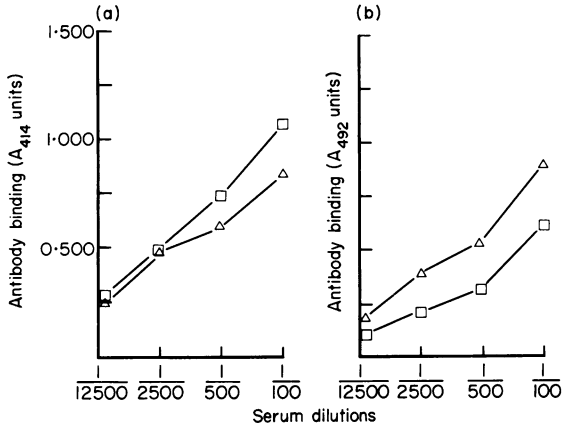

Fig. 1. Quantification of IdD23 idiotypes present in the serum of MRL-1pr/1pr (□—□) and BALB/c (△—△) mice. Plates coated with rabbit anti-IdD23 were incubated with the mouse sera, washed, and then incubated with either (a) rabbit anti-mouse Ig antibody labelled with peroxidase or with (b) rabbit anti-mouse IgM antibody labelled with β-galactosidase.

The syngeneic mouse anti-IdD23 antibody was found to react only with idiotypic determinants carried by IgM D23 in BALB/c sera. MRL sera did not react with this antibody. The interaction of IdD23 present in BALB/c serum with mouse syngeneic anti-IdD23 antibody was inhibited by pre-incubating BALB/c serum with rabbit anti-IdD23 antibody. This indicates that rabbit anti-IdD23 is able to react with the idiotypic determinants recognized by the syngeneic mouse anti-IdD23 antibody.

Idiotypic suppression. Groups of 20 BALB/c or MRL-1pr/1pr newborn mice were injected with rabbit or mouse anti-IdD23 antibody and the concentration of IdD23 determinants in all sera was assessed. Neither rabbit nor mouse anti-IdD23 antibody significantly modified the amount of IdD23 in 1 or 2 month old BALB/c or MRL mice. The offspring of BALB/c mice treated during pregnancy with rabbit anti-IdD23 antibody were examined at 1 and 2 months of age. The idiotopes recognized by mouse syngeneic anti-IdD23 antibody were suppressed in these mice but not in their littermates born to untreated mothers. This suppression was evident 1 month but not 2 months after birth (Fig. 2). No modification was noted for the determinants recognized by the rabbit anti-IdD23 antibody.

Antibody suppression in BALB/c mice. The sera of mice injected with anti-IdD23 mouse or rabbit antibody were examined for variations in natural autoantibody concentration using plates coated with myosin, transferrin, DNA and TNP_{25}/BSA. No differences were observed between treated and untreated mice. Furthermore, mice injected with anti-idiotypic antisera were immunized with all the above various antigens in complete Freund's adjuvant and the antibodies produced were measured. No differences were noted between untreated and injected mice.

Suppression of anti-DNA antibodies in MRL-1pr/1pr mice. Once the presence of IdD23 determinants was established in MRL sera, two groups of mice were treated with either rabbit anti-IdD23 antibody or with corresponding quantities of normal rabbit IgG. Anti-DNA antibody titres were examined by ELISA in sera obtained 1 month after birth and every 15 days thereafter for a period of 3 months.

No significant differences in the anti-DNA antibody titres were found between the two groups 1 month after birth. However, at week 6 mice treated with anti-IdD23 antibodies had significantly lower titres of anti-DNA antibodies. This significant difference persisted until week 10. Between weeks 12 and 16, the differences between the two groups of mice were still evident (Fig. 3). Given that IdD23 determinants are present in many natural polyspecific autoantibodies, we examined whether anti-IdD23 antibody treatment in MRL mice affected only anti-DNA antibodies or other antibodies as well (Cohen, Rapoport & Eisenberg, 1985). Using a panel of antigens including mouse

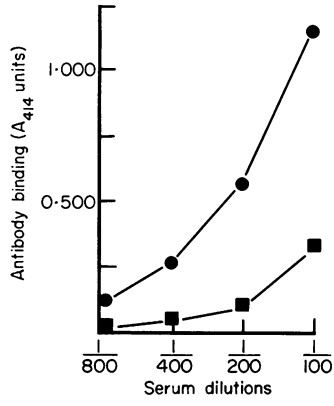

Fig. 2. Suppression of IdD23 idiotype recognized by the mouse syngeneic anti-idiotypic antibody after treatment of pregnant mice by rabbit IgG anti-IdD23 antibody. Experimental (■—■), and control (●—●) mice treated with normal rabbit IgG, were examined at 1 month after birth. The values given correspond to the mean values of seven sera tested individually, in each group.

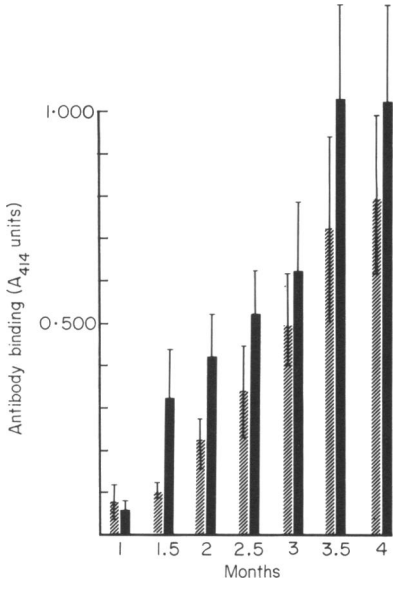

Fig. 3. Suppression of anti-DNA antibody titres in MRL-1pr/1pr sera (1:500 dilution) observed at various time intervals after birth. Mice were treated either with rabbit IgG anti-D23 antibody (■) or with normal rabbit IgG (■). The differences found only between 1·5 to 2·5 months are statistically significant ($P < 0{\cdot}05$, Student's t-test). Values given in each group correspond to the mean value of 20 mice sera tested individually.

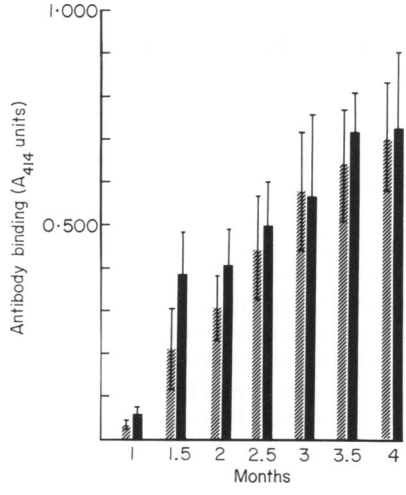

Fig. 4. Suppression of anti-TNP antibody titre in MRL-1pr/1pr sera (1:400 dilution) observed at various time intervals after birth. Mice were treated either with rabbit IgG anti-D23 antibody (■) or with normal rabbit IgG (■). Values given are as in Fig. 3.

actin, myosin, tubulin, albumin and transferrin and human albumin, transferrin and TNP_{25}/BSA, the sera obtained between 4 and 16 weeks after birth were tested by ELISA. A small difference in anti-TNP titres was observed (Fig. 4); this difference was statistically significant only for week 4 and week 6 samples ($P < 0{\cdot}01$). No difference was noted for all the other antibody titres.

DISCUSSION

The results of this study indicate that anti-idiotypic antibodies directed against natural autoantibodies may play a role in the regulation of the immune system. Apparently, this role is minor in normal mice perhaps because their regulatory system is intact and injection of anti-idiotypic antibodies cannot significantly perturb the established equilibrium. In contrast, anti-idiotypic antibodies have a more evident role in autoimmune MRL-1pr/1pr mice with signs of early immune dysregulation (Murphy & Roths, 1978).

The absence of idiotypic suppression in newborn BALB/c and MRL-1pr/1pr mice by administration of either the rabbit or the mouse anti-idiotypic antibody may be related to the ubiquity of natural autoantibody-producing cells in these mice. Indeed, it has been shown that such cells are present throughout life in normal mice as well as in hyperimmunized animals (Dighiero *et al.*, 1983; 1985; Guilbert *et al.*, 1985). Lack of idiotypic suppression after administration of anti-idiotypic antibodies in MRL-1pr/1pr mice has already been reported by other investigators who argued that this was due to the deficient immune system of these mice (Teitelbaum *et al.*, 1984). In these latter studies, however, idiotype expression in BALB/c mice was found to increase after treatment with anti-idiotypic antiserum. The difference between this result and our present findings may be related to differences in the quantity and/or specificity of the anti-idiotypic antibodies injected.

In the present work, the only idiotypic suppression noted was that of determinants recognized by the syngeneic mouse anti-idiotypic antiserum in offspring of pregnant mice injected with the rabbit anti-IdD23 antibody. This result can be explained if one considers that the rabbit anti-IdD23 antibody also recognizes the idiotopes recognized by the syngeneic anti-idiotypic antiserum. The suppression, observed only at 1 month after birth, could be the result of transient inactivation of cells producing these idiotopes. This concords with the notion that immature cells present in the fetus are more susceptible than mature cells to such inhibition (Accolla et al., 1977).

The autoimmune state of the MRL-1pr/1pr mouse is characterized by massive production of autoantibodies directed against DNA and other nuclear and cytoplasmic antigens (Murphy & Roths, 1978; Cohen, Rapoport & Eisenberg, 1985). In the present work, we have noted that injection of anti-IdD23 antibody resulted in a specific and significant decrease of anti-DNA antibody production, and in a slight decrease of anti-TNP antibody concentration. In this connection it should be underlined that interaction of anti-IdD23 antibody with D23 is specifically and completely inhibited by DNA (Lymberi *et al.*, 1985). The recent observations that murine monoclonal anti-DNA antibodies from autoimmune mice and polyclonal anti-DNA antibodies from SLE patients possess both anti-DNA and anti-TNP antibody function (Serban *et al.*, 1985; Matsiota *et al.*, 1987) may account for the decrease of anti-TNP antibodies also noted in this study.

In several studies of autoimmune animals, it has been shown that the *in vivo* production or passive administration of anti-idiotypic antibodies results in a decrease in pathogenic antibodies against thyroglobulin (Zanetti & Bigazzi, 1981) and DNA (Brown, Carey & Colvin, 1979). Similarly, it has been shown that the reaction of lupus sera with DNA can be inhibited by anti-idiotypic anti-DNA antibodies (Abdou *et al.*, 1981). Other authors (Hahn & Ebling, 1983) have also shown that the administration of an antibody to DNA bearing an idiotype occurring with high frequency in NZB/NZW F1 mice results in the suppression of the antibody response to double-stranded DNA as well as in suppression of nephritis in these same mice. These authors suggest that suppression was due to the presence of anti-idiotypic antibodies. However, in other studies, treatment of mice with anti-idiotypic anti-DNA antibodies was found to have no effect (Teitelbaum *et al.*, 1984). We think that these differences might be due to the origin of the anti-DNA antibodies used to prepare the anti-idiotypic antiserum. In the latter study, an anti-DNA antibody deriving from a pathological situation and probably of more restricted specificity was used, whereas we used a natural autoantibody with a broad specificity for the production of anti-idiotypic antibody.

The level at which anti-idiotypic antibody is exerting its effect remains to be established. Are only B lymphocytes or only T lymphocytes involved or both? We believe that such an analysis should be initiated only after monoclonal anti-idiotypic antibodies of known specificities are produced.

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