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Anti-idiotype and immunosuppressant treatment of murine lupus

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SUMMARY

The effect of the administration of a xenogeneic anti-idiotype antibody (anti-Id33) to a cross-reactive idiotype (Id33) present on anti-dsDNA antibody was examined in 6-week-old (NZB/NZW) F1 (BWF1) female mice. The administration of anti-Id33 led to a transient reduction in immunoglobulins expressing Id33, followed by a rise at 30 and 34 weeks that was significantly higher than in untreated mice ($P < 0.05$). Likewise, anti-dsDNA antibody levels were significantly higher at 10 and 18 weeks than in untreated mice ($P < 0.01$). No differences were seen in survival to 40 weeks, proteinuria or the severity of glomerulonephritis. Concurrent administration of cyclosporin A (CyA) with anti-Id33 markedly ameliorated glomerular injury and proteinuria and improved survival. By contrast, glomerular injury, proteinuria and survival were worse in mice treated with cyclophosphamide plus anti-Id33, compared with untreated mice. Neither CyA nor cyclophosphamide treatment, when given with anti-Id33 altered serum levels of anti-dsDNA, anti-ssDNA or Id33$^+$ immunoglobin, compared with untreated mice. The different effects of CyA and cyclophosphamide on T lymphocytes and their discrepant effects on glomerular injury when given with anti-Id33 in this model lead us to postulate a role for T lymphocytes in the glomerular injury of BWF1 lupus.

Keywords anti-idiotype immunosuppressant murine lupus

INTRODUCTION

Idiotypic regulation of the immune response as conceived by Jerne (1974) is now supported by a large volume of experimental data (reviewed by Bona, 1987). There is good evidence that antibody response to exogenous antigens can be suppressed by anti-idiotype antibodies (Hart *et al.*, 1972; Eichmann, 1974). Autoantibody production could be similarly regulated in autoimmune diseases such as systemic lupus erythematosus (SLE). It appears that the majority of anti-DNA antibodies in both mouse (Marion *et al.*, 1982; Rauch *et al.*, 1982) and human SLE sera (Shoenfeld *et al.*, 1983; Solomon *et al.*, 1983) bear cross-reactive idiotypes. At least some of these antibodies are potentially pathogenic (Ebling & Hahn, 1980) and attempts to suppress their production in murine lupus by the administration of idiotype or anti-idiotype antibodies have had varying effects: down-regulation of idiotype and anti-DNA antibody levels (Hahn & Ebling, 1983, 1984; Zouali *et al.*, 1985); no effect (Jacob & Tron, 1984); and up-regulation of idiotype and anti-DNA antibody levels (Teitelbaum *et al.*, 1984).

Recently, Mendlovic *et al.* (1988a) reported the induction of an SLE-like disease in normal C3H.SW mice by immunization with an anti-DNA antibody (carrying the 16/6 idiotype) indicating that under the appropriate circumstances, disease can be induced by antibodies that might under other circumstances be immunosuppressive. This uncertainty in outcome of idiotype manipulation raises problems for its possible application in the treatment of autoimmune disease. Here we have examined the effect of anti-idiotype administration with concurrent immunosuppressive therapy on the progression of disease in (NZB/NZW) F1 (BWF1) female mice. Our aims were to prevent the up-regulation of idiotype levels and of idiotype-negative but possibly pathogenic autoantibody production that may ensue with suppression of the major idiotype on autoantibodies (Hahn & Ebling, 1984). The immunosuppressive drugs cylosporin A (CyA) and cyclophosphamide were chosen, for two reasons. Firstly, because of their different modes of action which, although complex, can be summarized as follows: CyA inhibits T helper cell responses by inhibiting interleukin-2 (IL-2) production, thereby preventing the proliferation of activated T cells which express IL-2 receptors (Hess & Columbani, 1986) and cyclophosphamide inhibits polyclonal B cell activation and T suppressor cell function (Turk & Parker, 1982). Secondly, both CyA and cyclophosphamide are now being used in treatment of SLE patients, and it is important to know the effects of their interactions with a possible new therapy, since in the clinical setting it may be necessary to continue their use.

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MATERIALS AND METHODS

Animals

BWF1 female mice were obtained from Harlan Olac (Bicester, UK) and housed under standard laboratory conditions.

Monoclonal anti-dsDNA antibody

A monoclonal DNA-binding antibody (IgG2a,κ) was derived from the fusion of spleen cells from a 10-month-old diseased BWF1 female mouse and P3/NS1/1-Ag4-1 myeloma cells, as previously described (Morgan *et al.*, 1985a). This monoclonal antibody (MoAb 33) is specific for conformational determinants expressed on dsDNA and does not react with ssDNA (Morgan *et al.*, 1985a). MoAb 33 was purified from hybridoma culture supernatants by ammonium sulphate precipitation (45% saturation) followed by chromatography on a protein A/sepharose-4B column. The column was washed through with 0·1 M phosphate buffer and IgG bound to the column eluted with 0·1 M citrate/0·2 M phosphate buffer, pH 2·8, readjusted to neutral pH with saturated Tris base and then dialysed into phosphate-buffered saline (PBS; 0·15 M, pH 7·4)

Anti-idiotypic antibody to Id33

This was raised in sheep. One-hundred micrograms of MoAb 33 emulsified with an equal volume of Freund's complete adjuvant (Difco, Detroit, MI) were injected intravenously into a sheep on day 0, followed by another i.v. injection of 500 μg of MoAb 33 in normal saline on day 14. Two weeks later the sheep was plasmaphoresed. The IgG fraction of the sheep antiserum was purified by 45% ammonium sulphate precipitation followed by ion-exchange chromatography on DEAE/sepharose (Sigma Chemical Company, Poole, UK) and eluted with 0·03 M phosphate buffer. The sheep IgG preparation was absorbed to remove non anti-idiotypic antibodies by repeated passage through two affinity columns: (i) BALB/c mouse IgG coupled to CNBr-Sepharose-4B; and (ii) normal mouse IgG (Sigma) CNBr Sepharose-4B. The sheep anti-IgG was applied sequentially to columns (i) and (ii) and washed through with 0·1 M phosphate buffer. The fall-through fractions were collected and pooled. Bound immunoglobulins were eluted with 0·1 M citrate/0·2 M phosphate buffer, pH 2·8. The effectiveness of absorption was monitored by ELISA and the process was repeated until all antibody activity to normal mouse IgG was removed, while binding to the Id33 was retained. Two control IgG2aκ monoclonal antibodies were also used in the ELISA: 228, specific for ssDNA (Morgan *et al.*, 1985a); and N4A1, a non-DNA-binding monoclonal antibody.

Treatment of mice

Animals were assigned to groups of six to 10 and treated as follows. Purified anti-Id33 antibody was administered intraperitoneally at 6, 8 and 10 weeks of age in doses of 100 μg. Controls received normal sheep IgG similarly. Cyclophosphamide (60 mg/kg) was administered intraperitoneally, concurrently with each dose of anti-idiotypic antibody. CyA (20 mg/kg) was administered intraperitoneally on days −1, 1 and 2 with respect to each dose of anti-idiotype. Further control animals received cyclophosphamide alone, or CyA alone, or normal saline alone.

Urinary protein

Individual mice were housed in metabolic cages and 24-h urine samples were obtained at 2-weekly intervals. Protein content was assayed by the Biuret method (Pesce, 1974), modified for small volumes.

Antibodies to dsDNA

These were measured by ELISA as previously reported (Morgan *et al.*, 1985a), with slight modifications. All incubations were in duplicate and the plates washed three times with PBS/0·05% Tween (PBS-T) between incubations. Microtitre plates (Cooke M129A; Dynatech laboratories, Plochingen, FRG) were coated with poly-L-lysine (British Drug Houses, Poole, UK) at 50 μg/ml in PBS (0·15 M, pH 7·4) for 18 h at 4°C. Sequential incubations were carried out with: (i) S1 nuclease-treated dsDNA (calf thymus, highly polymerised DNA, type II, Sigma) (Ando, 1966) at a concentration of 5 μg/ml in PBS for 1 h at 37°C; (ii) test mouse sera (diluted 1/25 in PBS-T and 1% casein (PBS-T-C)) for 2 h at 4°C; (iii) sheep anti-mouse IgG alkaline phosphate conjugate (working dilution of 1/1000 in PBS-T-C) for 2 h at room temperature; and (iv) the enzyme substrate *P*-nitrophenyl phosphate (1 mg/ml in 10% diethanolamine buffer, pH 9·8) for 30 min at 37°C. The colour reaction was stopped with 3 M NaOH and the absorbance was read at 405 nm on a Titertek Multiscan reader (Flow Laboratories, Rickmansworth, UK). The results are expressed as absorbance values corrected for non-specific binding of conjugate to antigen-coated wells. The inter-assay coefficient of variation was 1·6–4·7% and the intra-assay variation was 5·2%, as determined by comparing the mid-point titres of a dilution curve of MoAb 33 included on each plate.

Antibodies to ssDNA

These were measured using a solid-phase ELISA as described above. ssDNA was prepared by heating the calf thymus DNA in PBS (0·15 M, pH 7·4) at 100°C for 15 min and chilling in an ice bath before use.

Binding site of anti-Id33 antibody

This was determined by the inhibition by dsDNA of binding of anti-Id33 to MoAb-33-coated plates in an ELISA. A range of concentrations of dsDNA (10–400 μg/ml, 0·15 M, pH 7·4) was incubated on MoAb-33-coated plates (2 μg/ml in 0·05 M carbonate/bicarbonate, pH 9·6, coating buffer) and then sequential incubations were performed with anti-Id33, alkaline-phosphatase-conjugated swine anti-sheep IgG and substrate as in the antibody ELISA.

ELISA for serum Id33$^+$ immunoglobulin

Serum levels of Id33$^+$ immunoglobulin were measured by the inhibition of binding of sheep anti-Id33 to MoAb-33-coated plates in an ELISA. First, plates were coated with purified MoAb 33 (2 μg/ml in 0·05 M carbonate/bicarbonate, pH 9·6) coating buffer by incubation for 18 h at 4°C, and then blocked with 2% casein/PBS for 1 h at 37°C. Aliquots of an equal volume mixture of test mouse sera (diluted 1/1000 in PBS-T-C) and anti-Id33 (at a concentration 1/40 giving 60% binding to MoAb 33), were pre-incubated for 18 h at 4°C, then added to the plate for 2 h at 4°C. Bound anti-Id33 antibody was detected by the incubation of alkaline-phosphatase-conjugated swine anti-sheep IgG (1/1000, BDS Biologicals, Birmingham, UK) for 2 h

at 37°C, followed by substrate as in the previous ELISA (30 min at 37°C). The absorbance was read at 405 nm on a Multiscan reader first blanked on the absorbance produced by the background binding of conjugate to MoAb-33-coated plates in the absence of anti-Id33. On each plate the following controls were included: (i) a set of doubling dilutions of MoAb 33 (1000–3·9 ng/ml) pre-incubated with anti-Id33 to give a standard inhibition curve; (ii) a negative control of normal BALB/c mouse serum pre-incubated with anti Id33; and (iii) a set of wells containing anti-Id33 alone to show the maximum MoAb 33/anti-Id 33 interaction. The results for each determination were expressed as the percentage inhibition of the Id/anti-Id reaction according to the formula

$$\frac{\text{OD without inhibitor} - \text{OD with inhibitor}}{\text{OD without inhibitor}} \times 100$$

A standard curve of % inhibition against Id33$^+$ immunoglobulin concentration was constructed and used to determine the Id33$^+$ immunoglobulin levels of the test sera. The inter-assay and intra-assay coefficients of variation were calculated from the concentrations of moAb 33 required for 50% inhibition of the MoAb 33/anti-Id33 reaction and were found to be 1·8–4·6% and 4·2% respectively.

Anti-Id33 assays

To detect anti-Id33 levels in the mouse sera, two assay systems were used:

Competitive Id/anti-Id assay. Microtitre plates were coated with MoAb 33 (18 h, 4°C) at a limiting concentration (1/80) that bound only 50% of the dilution of anti-Id33 used (determined by preliminary checkerboard titration). The plates were then washed three times with PBS-T buffer between sequential incubations of: (i) 2% casein–PBS for 1 h at 37°C; (ii) mouse sera (diluted 1/25 in PBS-T-C) in duplicate, 1 h, 37°C; (iii) anti-Id33 (diluted 1/40 in PBS-T-C) for 1 h at 37°C; (iv) swine anti-sheep IgG alkaline-phosphatase conjugate (1/1000 in PBS-T-C) 2 h, room temperature; and (v) *P*-nitrophenyl phosphate substrate, 30 min, 37°C.

The absorbance was read and results expressed as in the previous ELISA.

Idiotype capture assay. Mouse sera, diluted 1/25 in coating buffer, were incubated in duplicate on microtitre plates for 18 h at 4°C. The plates were washed three times with PBS-T buffer, then incubated with 2% casein-PBS for 1 h at 37°C. After further washing, a range of dilutions of ^{125}I-radiolabelled MoAb 33 (labelled by the chloramine T method, as described by Greenwood, Hunter & Glover (1963), to a specific activity of $2{\cdot}6 \times 10$ ct/min per μg TCA precipitable protein) was added. After incubating for 2 h at 37°C, the plates were washed and dried, the wells were cut, and bound radioactivity was counted in a gamma counter (NE 1600, Nuclear Enterprises). A negative control of solid-phase normal BALB/c mouse immunoglobulin and a positive control of sheep anti-Id33 immunoglobulin were included on each plate.

IgM rheumatoid factor assay

Microtitre plates were incubated with normal pooled mouse IgG (Sigma) at a concentration of 5 μg/ml in coating buffer for 2 h at 37°C, followed by mouse sera diluted 1/25 in PBS-T-C (2 h, 37°C) then by sheep anti-mouse IgM alkaline-phosphatase conjugate (diluted 1/1000 in PBS-T-C) for 2 h at 37°C, and finally *p*-nitrophenyl phosphate substrate for 30 min at 37°C, with washing between each stage. The results were expressed as absorbance values (OD at 405 nm). Normal mouse sera were used as negative controls.

Histology

Kidneys were obtained from animals that died or were killed by ether anaesthesia and cervical dislocation. Portions of kidney were fixed in 10% formol saline, sectioned at 4 μm and 2 μm, and stained with haematoxylin and eosin and periodic acid silver, respectively. Glomerular bound IgG was detected by an indirect immunoperoxidase technique using sheep anti-mouse IgG and peroxidase-conjugated swine anti-sheep IgG (BDS Biologicals). Glomerular deposition of Id33$^+$ immunoglobulin was detected by incubating renal sections with sheep anti-Id33 antibody, followed by peroxidase-conjugated swine anti-sheep IgG. Coded sections were examined by microscopy without prior knowledge of the experimental protocol.

Statistical analysis

Differences between the experimental groups were analysed using the Wilcoxon rank sum test. Data on survival times were analysed using the Log rank test (Peto *et al.*, 1977).

RESULTS

Characterization of anti-Id33

The binding affinities of the anti-Id33 antiserum are summarized in Table 1. After absorption, this reagent bound to MoAb 33 but not to isotype-matched mouse monoclonal IgG2aκ ssDNA binding antibody or to normal BALB/c mouse IgG. In an inhibition assay, a concentration of dsDNA of 400 μg/ml only inhibited 23·2% of the binding of anti-Id33 to MoAb 33, and it is therefore likely that this anti-idiotype antiserum was directed mainly against framework antigens.

Anti-Id33 treatment

Anti-Id33 treatment improved survival to 80%, compared with untreated controls (60%) but the difference was not significant ($P > 0{\cdot}05$) (Fig. 1a). The time of onset of proteinuria and its cumulative incidence were comparable in these two groups (Fig. 2a). The severity of glomerular damage was comparable in the anti-Id33 and untreated groups (Table 2). Mean levels of serum anti-dsDNA antibodies were higher in the anti-Id33 treated groups when compared with untreated animals from 10 to 40

Table 1. Binding affinities of anti-Id33

	Antibody mid-point titres			
Anti-Id33	MoAb33	MoAb228	MoAbN4A1	BALB/c mouse IgG
Unabsorbed	400	525	1600	400
Absorbed	150	0	0	0

Antibody mid-point titre is defined as the reciprocal dilution at 50% maximum optical density in a direct binding assay.

MoAb228, mouse monoclonal IgG2aκ ssDNA-binding antibody; MoAbN4A1, mouse monoclonal IgG2aκ non-DNA-binding antibody.

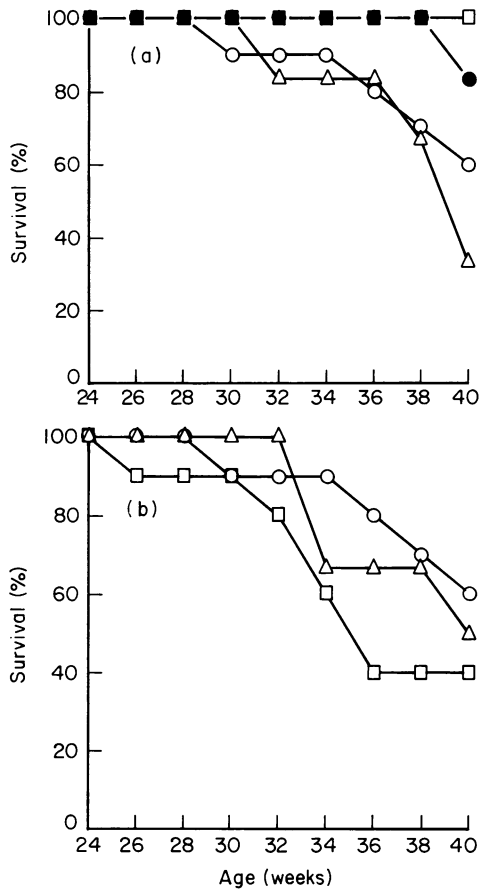

Fig. 1. Survival curves for the different treatment groups: (a) ●, anti-Id; □, anti-Id + CyA; △, anti-Id + cyclophosphamide; ○, no treatment; (b) □, CyA; △, cyclophosphamide; ○, no treatment.

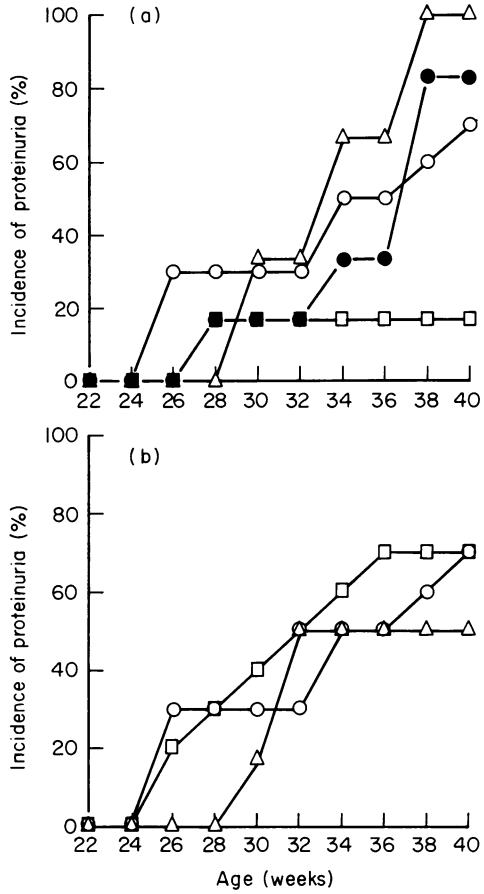

Fig. 2. Cumulative incidence of proteinuria for the different treatment groups (per cent of mice with proteinuria > 10 mg/24 h with increasing age). Mice that died with high proteinuria were included in the calculation of incidence at the next time-point. (a) ●, anti-Id; △, anti-Id + cyclophosphamide; □, anti-Id + CyA; ○, no treatment; (b) □, CyA; △, cyclophosphamide; ○, no treatment.

weeks (Fig. 4) and these differences were significant at 10 and 18 weeks ($P < 0{\cdot}01$). At 26 weeks and 30 weeks two out of six anti-Id-treated animals had anti-dsDNA antibodies greater than 2 s.d. above the mean anti-dsDNA levels of the untreated animals and this rose to three out of six and four out of six at 34 and 38 weeks, respectively. Anti-ssDNA antibody levels were comparable in these two experimental groups as well as in the other groups throughout the 40 weeks (data not shown). A transient reduction in $Id33^+$ immunoglobulin levels occurred in the anti-Id33-treated group at 18 weeks ($P < 0{\cdot}05$) as compared with the untreated group (Fig. 3); subsequently $Id33^+$ immunoglobulin levels were significantly higher in anti-Id33 treated group at 30 and 34 weeks ($P < 0{\cdot}05$).

Anti-Id33 plus CyA treatment

All mice treated with anti-Id33 plus CyA survived to 40 weeks, as compared with 60% of untreated mice (Fig. 1a). Only one out of six had significant proteinuria (> 10 mg/24 h) as compared with 70% of the control group (Fig. 2a). Glomerular damage was minimal in mice treated with anti-Id33 plus CyA who had early mesangial proliferative glomerulonephritis with minor mesangial deposits of IgG at 40 weeks. In contrast, untreated mice had an advanced to end stage mesangial proliferative and membranous nephropathy with mesangial and capillary deposition of IgG (Table 2). Anti-dsDNA (Fig. 4) and $Id33^+$ immunoglobulin levels (Fig. 3) were comparable at all stages in the mice treated with anti-Id33 plus CyA and in the untreated group.

Anti-Id33 plus cyclophosphamide

Only 33% of the mice treated with anti-Id33 cyclophosphamide survived to 40 weeks, compared with 60% of the untreated mice (Fig. 1a). All mice in the anti-Id33 plus cyclophosphamide group had significant proteinuria at 40 weeks, compared with 70% of the untreated mice (Fig. 2a).

The mice treated with anti-Id33 plus cyclophosphamide had severe and advanced-to- end-stage glomerular damage with severe global glomerular sclerosis (Table 2). There were no differences in the anti-dsDNA antibody levels (Fig. 4), anti-ssDNA antibody levels (data not shown), and $Id33^+$ immunoglobulin levels (Fig. 3) between these two groups of mice.

CyA alone, cyclophosphamide alone, normal sheep IgG alone, no treatment groups

Other than a lower incidence of proteinuria at 40 weeks of 30% in the mice treated with cyclophosphamide alone, there were no major differences between mice treated with CyA alone and cyclophosphamide alone, compared with untreated mice in

Table 2. Renal histology of animals in each group at stages of glomerular damage, and glomerular deposits of Id33+ immunoglobulin

Group	n	Stage of glomerular lesions			Glomerular deposits†		
		Early	Advanced	End stage	IgG M	IgG C	Id33+ Immunoglobulin
Anti-Id33 alone	6	1	5	0	+/++	+	4/5
Anti-Id33+CyA	6	6	0	0	+	−	1/6
Anti-Id33+CyP	6	0	0	6*	+	+	1/6
No treatment	9	2	5	2	+/++	+	3/6
Normal sheep IgG	6	1	4	1	+/++	+	nd
CyA alone	10	3	7	0	nd	nd	nd
CyP alone	6	2	4	0	nd	nd	nd

*Advanced–End stage.
†M, mesangial; C, capillary; −, negative; +, positive; ++, strongly positive; ND, not done.
CyA, cyclosporin A; CyP, cyclophosphamide.

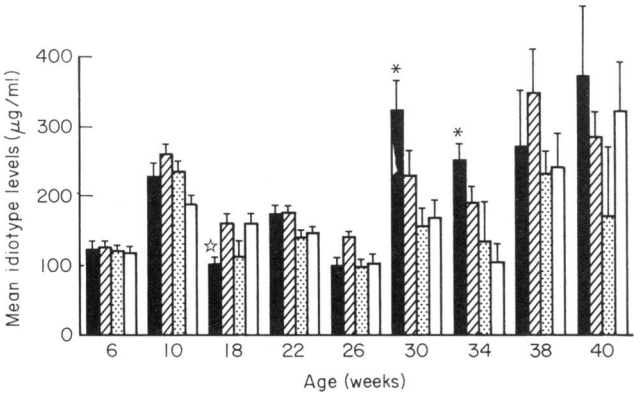

Fig. 3. Id33+ immunoglobulin levels (μg/ml) for treatment groups at different ages up to 40 weeks: ■, anti-Id; ▩, anti-Id+CyA; ▨, Anti-Id+cyclophosphamide; □, no treatment. Mean+s.e.m. *$P<0.05$.

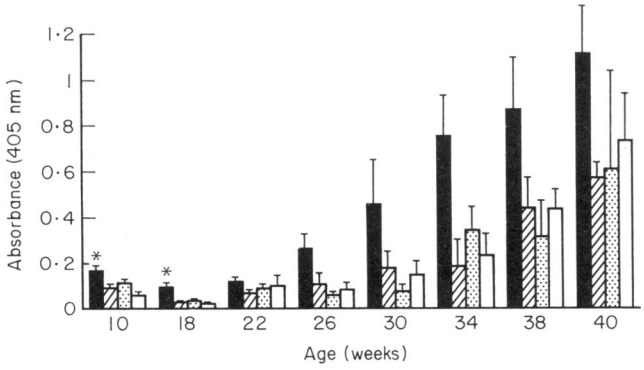

Fig. 4 Anti-dsDNA antibody levels (absorbance at 405 nm) for treatment groups at different ages up to 40 weeks: ■, anti-Id; ▩, anti-Id+CyA; ▨, Anti-Id+cyclophosphamide; □, no treatment. Mean± s.e.m. *$P<0.01$.

survival (Fig. 1b), proteinuria (Fig. 2b), anti-dsDNA antibody (Fig. 4), anti-ssDNA antibody (data not shown), Id33+ immunoglobulin levels (Fig. 3), and the histological severity of glomerulonephritis (Table 2). No differences were seen between mice treated with normal sheep IgG and untreated mice (data not shown).

Serum anti-Id33 antibody levels

Using both assays no sera from untreated mice or mice in any of the treatment groups had detectable anti-Id33 antibodies.

Rheumatoid factor

No sera from untreated mice or any of the treated groups had rheumatoid activity.

Renal histology

Table 2 summarizes the histology of each animal in the different experimental groups either when they died or at 40 weeks. Mice in the untreated control group had a mesangial proliferative and membranous glomerulonephritis with IgG deposits that were predominantly mesangial. Three out of six mice in this group had glomerular deposits of Id33+ immunoglobulin. In contrast, mice treated with anti-Id33 plus CyA had an early and mild mesangial proliferative glomerulonephritis with scanty mesangial deposits of IgG. Only one out of six mice in this group had glomerular deposits of Id33+ immunoglobulin. All mice treated with anti-Id33 plus cyclophosphamide had advanced-to-end-stage glomerular disease with marked global glomerular sclerosis and minimal glomerular deposits of IgG. Only one out of these six mice had glomerular deposits of Id33+ immunoglobulin.

DISCUSSION

Current treatment of SLE with steroids and immunosuppressant drugs (azathioprine, cyclophosphamide and CyA) is unsatisfactory because of the well-established toxicity of these agents. The possibility of down-regulating potentially pathogenic autoantibodies in this disorder with anti-idiotypic antibodies is, therefore, attractive. Hahn & Ebling (1983, 1984) have

shown that cross-reactive idiotypes expressed by DNA binding antibodies can be suppressed in BWF1 mice by the administration of idiotype or anti-idiotype antibody. This suppression led to a delay in the onset of nephritis and of death. These studies highlighted potential problems inherent in idiotypic manipulation, namely the development of target idiotype-negative but potentially pathogenic dsDNA-binding antibodies, and also the up-regulation of idiotype-bearing autoantibodies.

The anti-Id33 reagent used in this study appeared to detect both binding site and framework determinants, and the extent to which these different anti-idiotypic antibodies contributed to the stimulatory or suppressive effects described is not known. Most idiotypes associated with DNA-binding antibodies and described so far in the literature are public or cross-reactive, and many of them are in or include the binding site (Isenberg & Staines, 1990). Our results are therefore in accord with others (Hahn & Ebling, 1983, 1984; Epstein *et al.*, 1987; Mahana, Guilbert & Avrameas, 1987), indicating that cross-reactive anti-idiotype reagents are potentially immunoregulatory. Given the extensive idiotypic sharing among DNA-binding autoantibodies, there are reasonable prospects for clinical manipulation by therapy with common anti-idiotype antibodies.

It is not obvious from these experiments that either MoAb 33 or other antibodies expressing Id33 are pathogenic. It is known, however, that MoAb 228, a DNA-binding antibody from an MRL mouse, is nephrotoxic (Lake & Staines, 1988), and that MoAb 33 reacts weakly with anti-idiotype antibodies raised against MoAb 228 (Staines *et al.*, unpublished results). Thus linkage of the idiotype of MoAb 33 to other pathogenic antibodies is at least implied by the present studies. In an analogous way, treatment of lupus mice with anti-Id.F-423 or anti-Id.IV-228 antibodies will ameliorate disease and depress the levels of immunoglobulins bearing not only the homologous idiotypes but also, in both cases, immunoglobulins expressing Id. V-88 (manuscript in preparation). This idiotype defined originally on a DNA-binding antibody (V-88) of different fine specificity, but from the same animal as MoAb 33 (Morgan *et al.*, 1985a), expresses the human Id16/6 that is associated with human lupus diseases (Morgan *et al.*, 1985b). These observations implicate Id33 in the pathogenic process of murine lupus.

Here, the administration of anti-Id33 antibodies significantly increased Id33-bearing immunogloblin levels following a transient early reduction, and also increased anti-dsDNA antibody levels compared with untreated mice. This enhancement of idiotype and anti-DNA antibody levels after anti-idiotype antibody administration has also been reported in MRL/++ and BALB/c mice (Teitelbaum *et al.*, 1984). The concurrent administration of CyA or cyclophosphamide together with anti-Id33 to BWF1 mice resulted in contrasting effects. Firstly, the administration of anti-Id33 plus CyA was associated with a marked amelioration of disease with 100% survival at 40 weeks, little proteinura and minimal glomerular damage. Secondly, the administration of anti-Id33 plus cyclophosphamide was associated with worse proteinuria, an increased severity of glomerulonephritis and worse survival, compared with untreated mice. Thirdly, these differences in the severity of glomerulonephritis were not associated with observable differences in serum levels of Id33 immunoglobulin, anti-dsDNA antibodies or anti-ssDNA antibodies.

Glomerular deposits of IgG and of Id33$^+$ immunoglobulin were scanty in the mice treated with anti-Id33 plus cyclophosphamide. The most likely explanation for this is that the advanced nature of their renal disease with severe global glomerular sclerosis masked the presence of immune deposits. Id33$^+$ immunoglobulin was found in the glomeruli of three out of six untreated mice and it seems likely that this cross-reactive idiotype is involved in the pathogenesis of the glomerulonephritis. In keeping with this observation, only one out of six mice treated with anti-Id33 and CyA had glomerular deposits of Id33$^+$ immunoglobulin. All these mice had only minor glomerular lesions with an early mesangial proliferative lesion. Isenberg & Collins (1985) reported glomerular deposition of a cross-reactive anti-DNA antibody idiotype, 16/6 Id, in the glomeruli of 11 out of 26 renal biopsies from patients with SLE.

A major observation of our study was the amelioration of glomerulonephritis and lack of glomerular Id33$^+$ immunoglobulin deposition in animals treated with anti-Id33 and CyA. Since serum levels of anti-dsDNA antibodies and Id33$^+$ immunoglobulin were comparable to those seen in control animals who developed severe glomerulonephritis, the effects of the anti-ID plus CyA treatment are best explained at a level other than that of the B lymphocyte. T cells are found in the glomeruli of experimental animals and humans with anti-GBM and immune-complex-mediated glomerulonephritis (Holdsworth *et al.*, 1980; Hooke *et al.*, 1984; Caligarris-Cappio *et al.*, 1985; Nolasco *et al.*, 1987). There are also reports of T cell playing a role in the recruitment of macrophages into the glomeruli of experimental antibody-induced nephritis (Bhan *et al.*, 1979; Tipping, Neal & Holdsworth, 1985; Boyce, Tipping & Holdsworth, 1986). In order for T helper cells to recruit macrophages they need to be activated and this requires recognition of antigen in association with class II MHC antigens. Glomerular endothelial cells may provide the class II MHC antigen requirement, since endothelial cells are known to express these antigens upon exposure to interferon-gamma (Pober *et al.*, 1983). Furthermore, Wagner, Vetto & Burger (1984) showed that cloned endothelial cells were capable of presenting antigen in association with class II MHC antigens to T cells *in vitro*.

There is evidence that idiotype T cells recognize idiotypic determinants in association with class II antigens as for any other antigen (Jorgensen & Hannestad, 1980; Yamamoto *et al.*, 1983). Such idiotype-specific T cells could recognize idiotype within glomeruli. In patients with SLE the 16/6 idiotype on monoclonal anti-dsDNA IgM antibody has been shown to be capable of activating T cells to secrete helper T cell factors (Mendlovic *et al.*, 1988b) and two of us (N.A.S. and A.M., unpublished) found elevated anti-DNA anti-idiotype responses in T cells of *lpr* mice compared with MRL+/+ or BALB/c mice.

Our data highlight some of the difficulties in attempting to manipulate on ongoing autoimmune disorder by the administration of anti-idiotype antibody. The different effects of CyA and cyclophosphamide when administered together with anti-idiotype antibody suggest that idiotype recognising T lymphocytes may be important in the development of glomerulonephritis in BWF1 lupus.

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