Suppression of NZB/NZW Murine Nephritis by Administration of a Syngeneic Monoclonal Antibody to DNA

POSSIBLE ROLE OF ANTI-IDIOTYPIC ANTIBODIES

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ABSTRACT Suppression of circulating antibodies to double-stranded DNA was achieved in NZB/NZW f_1 female mice by repeated administration of an IgG2a monoclonal antibody to DNA. Deaths from nephritis were delayed; glomerular deposition of IgG and of the cationic IgG DNA antibodies characteristic of murine lupus nephritis were diminished. Quantities of circulating antibodies to single-stranded DNA were not reduced compared with untreated or IgG myeloma-treated control mice. Antibodies directed against the monoclonal anti-DNA appeared in the circulation of treated mice after three inoculations of the idiotype. Those antibodies did not react with another monoclonal anti-DNA of the same allotype. One monoclonal anti-idiotypic antibody was obtained in hybridoma cultures derived from a spleen of a treated mouse. Cross-reactive or common idiotypes were found in 30–50% of NZB/NZW f_1 sera and monoclonal DNA antibodies. Deletions of portions of the spectrotype of antibodies to DNA were found in sera containing anti-idiotypic antibodies, suggesting suppression of clones producing antibodies with isoelectric points similar to that of the immunizing idiotype. Deletions of some of the anti-idiotypic antibodies also occurred as the mice aged. Rheumatoid factors were not detectable in any sera.

Therefore, administration of an antibody to DNA bearing an idiotype occurring with high frequency in NZB/NZW f_1 females resulted in relatively specific suppression of the antibody response to double-stranded DNA, as well as suppression of nephritis. Reduction of anti-DNA synthesis by anti-idiotypic antibodies may have been an important suppressive mechanism. Experiments are in progress to test this hypothesis.

INTRODUCTION

It is generally agreed that antibodies to DNA, as well as to other nuclear and cytoplasmic antigens, participate in the pathogenesis of systemic lupus erythematosus, both in human and murine disease. Therefore, investigation of the characteristics of DNA antibodies which render them pathogenic has been extensive. In human and murine disease, DNA antibodies of the IgG class with the ability to fix complement have been associated with clinically active disease and with immune complex glomerulonephritis (1, 2). We have suggested that subpopulations of IgG antibodies to DNA that bear a cationic charge are more nephritogenic than other populations (3). Whether antigenic specificities of DNA antibodies influence pathogenicity remains controversial (4–7).

The advent of monoclonal antibody technology, introduced by Kohler and Milstein (8), has permitted a reassessment of the characteristics of DNA antibodies and has provided a new tool for disease modification in animal models of systemic lupus erythematosus. We have used a murine monoclonal antibody to DNA to test the hypothesis that DNA antibody synthesis might be reduced by maintaining mice in a state of antibody excess. We now report that repeated administration of a murine monoclonal IgG antibody to dsDNA resulted in suppression of circulating antibodies to

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dsDNA (but not of anti-ssDNA) and of immune glomerulonephritis in NZB/NZW female mice. Suppression was associated with appearance of antibodies specific for the injected IgG and with deletion of circulating anti-DNA with isoelectric points similar to that of the administered idiotype (Id).[1] Therefore, in these experiments, suppression of anti-DNA synthesis may have resulted at least in part from the production of anti-idiotypic antibodies (anti-Id), and we may have achieved relatively specific suppression of an undesirable immune response.

METHODS

Mice. NZB × NZW f_1 mice were bred from stock parental colonies maintained at Washington University and permitted ad lib. food and water without antibiotic supplementation. Only females were used in these studies. Littermates were randomized into each of three treatment groups.

Monoclonal antibodies and anti-Id. Our methods for production of monoclonal antibodies have been described in detail previously (9) and are modified from the original techniques of Kohler and Milstein (8). Briefly, 10^8 spleen cells from female NZB/NZW f_1 mice with circulating antibodies to DNA were fused with 10^7 murine myeloma cells (nonsecreting MOPC-21, line Sp 2/0-Ag14) in polyethylene glycol and propagated in 24-well culture dishes containing hypoxanthine/aminopterin/thymine selection medium. Supernatants from confluent monolayers were screened for antibodies to dsDNA in a polyethylene glycol-modified Farr assay, and positive cultures were cloned in soft agar over feeder layers.

One clone obtained in this manner and designated BWdsl was used to modify disease in these experiments. BWdsl was purified as IgG from BALB/c ascites fluid by column chromatography on DEAE-cellulose. In preparations of IgG used in these experiments, BWdsl composed 30–35% of the total Ig, as determined by focusing the monoclonal protein in polyacrylamide gels, eluting the bands, and determining protein content by optical density at 280 nm. BWdsl is an IgG2a kappa, which after isoelectric focusing in 5% polyacrylamide gel and precipitation with sodium sulfate forms four bands from pH 6.8 to 7.2 (Fig. 3). These bands bound calf thymus dsDNA (purified by endonuclease digestion) after gel overlay with ^{125}I-dsDNA, and in a modified Farr assay (71% binding of 40 ng of dsDNA by 1 μg of BWdsl) and in enzyme-linked immunosorbent assay (ELISA) solid-phase assay (titer of 1:64 with 8.2 μg of antibody incubated with 100 ng of calf thymus dsDNA fixed to a well; titer of 1:16 when ssDNA was the substrate). Since BWdsl reacted more strongly with ds than with ssDNA it was designated as an anti-dsDNA. Reactivity with dsDNA was consistently stronger in fluid phase tests than in solid phase, suggesting that more secondary structures and/or more single-stranded regions of the antigen were available in fluid phase.

LPC-1, an IgG2a kappa monoclonal immunoglobulin derived from a BALB/c myeloma with antibody activity against phosphorylcholine, served as a control. Ascites fluid containing LPC-1 IgG was a gift from Dr. Joseph Davie, Department of Microbiology and Immunology, Washington University; LPC-1 constituted 30–35% of the total IgG, similar to BWdsl. LPC-1 focused between pH 7.0 and 7.2. It did not bind ds or ssDNA after focusing and precipitation in gels, or in the Farr assay, but a low level of reactivity was seen in the ELISA assay (titer of 1:16 with 100 ng of dsDNA and 14 μg of IgG, titer of 0 with 100 ng of ssDNA and 279 μg of IgG).

Heterologous anti-idiotypic sera were produced by hyperimmunization of LPJ mice with BWdsl (Id). LPJ mice, chosen for a major IgGCH difference (LPJ are b; NZB/NZW are d) to maximize immunogenicity of BWdsl, were inoculated with 100 μg of Id IgG in CFA, and boosted with 100 μg at 4 and 8 wk. Two of six mice made circulating anti-Id; their spleens were used to immortalize clones of anti-Id in hybridoma cultures. We succeeded in isolating one clone, an IgGl with a pI of 6.2 (aBWdsl-L). Both aBWdsl-L and heterologous LPJ mouse sera were absorbed with young premorbid NZB/NZW f_1 IgG fixed to sepharose via cyanogen bromide, and served as sources of anti-Id. The absorbed anti-Id bound to BWdsl in solid-phase assays, but not to BWds2, an IgG2a kappa monoclonal anti-DNA derived from a NZB/NZW f_1 spleen different from the source of BWdsl, and with a different isoelectric point (6.0–6.2).

One monoclonal murine anti-Id was obtained from a hybridoma derived from the spleen of a NZB/NZW f_1 female (repeatedly inoculated with BWdsl), aged 25 wk. This antibody, designated aBWdsl-B1, reacted with BWdsl in solid-phase assay but not with BWds2. All binding activity was contained in the Fab portions of the polyclonal and monoclonal anti-Ids. Fab fragments were derived from ascites fluid IgG by the method of Spring and Nisonoff (10).

Study design. NZB/NZW f_1 female littermates were divided into three groups, each containing 12 mice. Treatment was begun at weaning, when the mice were 4–6 wk old. Treatment was continued until all mice were dead. Group A received intraperitoneal injections of BWdsl (IgG purified from ascites fluid) 100 μg once every 2 wk. In preliminary studies, ^{125}I-labeled BWdsl was injected intraperitoneally into young NZB/NZW mice; 85% of the inoculum could be found in circulation and tissues within 5 min: The half-life was 2 d, calculated by inoculating 53 × 10^6 cpm of ^{125}I-BWdsl into two treated and one untreated NZB/NZW females. Counts per minute in serum were measured at 0 and 3 h, then on days 1, 2, 4, 7, 10, and 15. The half-life was the same in treated and untreated mice. Group B received 100 μg of LPC-1 intraperitoneally once every 2 wk. Group C was untreated. The mice were studied daily for survival and every 2 wk (just before inoculation) for weight and proteinuria: Heparinized blood was obtained from the retroorbital plexus for measurement of urea nitrogen levels, and plasma was stored at −70°C for later analysis. Proteinuria was estimated by catching a drop of urine on Albustix (Ames Div. Miles Lab., Inc.); urea nitrogen levels were estimated by applying a drop of whole blood to Azostix (Ames Div. Miles Laboratories Inc., Elkhart, IN). Plasma was assayed later for antibodies to dsDNA and ssDNA (ELISA technique), for the spectrotype of DNA antibodies present (by isoelectric focusing), for rheumatoid factors, for Id and anti-Id. Techniques are detailed below.

20 additional NZB/NZW f_1 females were divided into groups A, B, and C and treated as detailed above from age 6 to 27 wk. They were killed at that time; two spleens from BWds-1-treated mice were used as fusion partners to produce monoclonal anti-Id (the source of aBWdsl-B1). Kidneys from these mice were snap-frozen in dry ice and acetone for later analysis of glomerular eluates. These mice were used only to provide renal tissue and spleens for fusion. They are

[1] *Abbreviations used in this paper:* anti-Id, anti-idiotypic antibodies; Id, idiotype.

not included in data regarding survival, incidence of antibodies to DNA, or incidence of anti-idiotypes.

Tests for antibodies to DNA. Three methods were used to study sera from test mice—a modified Farr assay, isoelectric focusing of IgG in slab gels followed by overlay with radio-iodinated DNA substrates, and an ELISA solid-phase assay. The DNA used in all assays was from calf thymus. For the Farr assay and gel overlay, ^{125}I-DNA was purchased from New England Nuclear (Boston, MA), where it was treated with endonuclease. We have studied several batches of this material by BNDC chromatography and have found no free ssDNA, ~40% nicked dsDNA, and 60% "pure" dsDNA. For the ELISA assay, DNA was purchased from Sigma Chemical Co. (St. Louis, MO) treated with endonuclease (Sigma Chemical Co.) and reprecipitated in 95% alcohol, after the method of Kirby (11). Therefore, the antigen we refer to as dsDNA contains some ss nicks, but no free ssDNA. Heat-denaturation of this antigen provided ssDNA.

We have previously described our polyethylene glycol-modified Farr assay (3), which is based on the technique of Riley et al. (12). Because it was quantitatively less sensitive than the ELISA assay, the analyses of serum antibodies in test mice presented here were measured by the ELISA method.

The method for detecting serum IgG antibodies to DNA by isoelectric focusing of 20 μl of serum (with 3 M urea) in 5% polyacrylamide vertical slab gels containing urea, precipitating the antibody bands in sodium sulfate, overlaying with ^{125}I-dsDNA, washing, and detecting antigen binding after x-ray film overlay is based on the technique of Briles and Davie (13); our modifications have been reported previously (3). This yields qualitative but not precise quantitative information about the spectrotypes of IgG anti-DNA present in an individual serum.

In the ELISA method, 100 μg of purified calf thymus dsDNA or denatured ssDNA were linked to methylated bovine serum albumin (Sigma Chemical Co.), then 150-μl vol were incubated in 96-well polyvinyl plates (Costar Co., Cambridge, MA) overnight at 4°C. The wells were then filled with 150 μl of nonimmune rabbit gamma globulin, 0.5 mg/ml, and incubated at 37°C for 30 min, then at 4°C overnight. Wells were washed extensively with phosphate-buffered saline (PBS) containing 0.05% Tween 80, pH 7.4. To assay for IgG anti-DNA, 150 μl of test mouse serum, serially diluted from 1:20 to 1:10,240, using 2.5 mg/ml bovine serum albumin in PBS pH 7.4, was added to plates containing either ds or ssDNA and incubated at 37°C for 45 min, then at 4°C overnight. Plates were washed with PBS/Tween, then rabbit anti-mouse IgG conjugated alkaline phosphatase (Miles Laboratories, Inc.) was added to each well and incubated for 50 min at room temperature, then at 37°C for 30 min. After washing with PBS/Tween, the enzyme substrate was added. After 30 min, the color development was blocked with 3 N NaOH and measured in a micro ELISA automatic reader (Dynatech Laboratories, Inc. Alexandria, VA). We preferred this methodology to several others tested, including DNA fixed directly to the polyvinyl plates and to other types of solid substrates, because we obtained larger and more reproducible quantities of both ds and ssDNA fixed to the plates, as well as lower background readings.

Rheumatoid factor testing. Sera from mice receiving no treatment or repeated injections of monoclonal IgG were tested for anti-IgG in a solid-phase assay. Heat-aggregated normal mouse IgG (Cappel Laboratories, Inc., Cochranville, PA), treated at 63°C for 30 min and cooled in ice to obtain aggregates, was labeled with ^{125}I by the chloramine T method (14) and added to each well of 96-well polyvinyl plates at a concentration of 100 ng/well. Serial dilutions of test sera were first incubated in these wells at 37°C for 1 h, then overnight at 4°C. Plates were incubated at room temperature for 2 h, followed by washing in PBS/Tween. The plates were washed extensively and each well cut out for determination of counts per minute in a gamma counter. Goat anti-mouse serum (200 μg/ml), served as a positive control and gave a value of 20,930 cpm; normal mouse serum was a negative control and gave a value of 257 cpm.

Tests for idiotype and anti-idiotypic antibodies. Individual mouse sera were examined for the presence of Id, for IgG bearing cross-reactive idiotypes and for anti-Id by two methods. First, heat-inactivated, undiluted test sera were coated onto the wells of 96-well polyvinyl plates starting at a concentration of 150 μl/well (~150 μg/ml of IgG). After washing, ^{125}I-labeled BWds1 (Id) or anti-Id were added to each well at concentrations ranging from 0.1 to 10 μg/ml. After incubation at 37°C for 45 min, then at room temperature for 2 h and washing, wells were cut and measured for counts per minute.

Secondly, IgG antibodies in test sera were separated and concentrated by isoelectric focusing in 5% polyacrylamide-urea gels, as for DNA antibodies. After precipitation of the antibodies, gels were overlaid with ~1 μg/lane of ^{125}I-labeled Id (BWds1), ^{125}I-labeled heterologous LPJ anti-Id, or 1 μg/lane of the monoclonal murine antibody aBWds1-L. After incubation at 37°C for 1 h, gels were washed and overlaid with Kodak SB-5 film for 1–3 d (Eastman Kodak Co., Rochester, NY); the film was subsequently developed. Only this method was successful in detecting Id-anti-Id interactions in sera. Id was most frequently detected by reaction of focused sera or monoclonal antibodies with aBWds1-L. When aBWds1-L was purified by DEAE chromatography, or by elution from polyacrylamide gels after focusing, most of its reactivity with Id (BWds1) was destroyed. However, radioiodination of ascites fluid containing aBWds1-L, followed by focusing and elution, showed that 96% of the iodine label was on the monoclonal IgG. Therefore, the sensitivity of the Id-anti-Id assay was determined by reacting ^{125}I-ascites-aBWds1-L with BWds1 highly purified by elutions from focusing gels and bound to polyvinyl plates, and with focused NZB/NZW sera in which quantities of Id plus antibody could be estimated visually by the numbers of bands precipitated by Na_2SO_4. In the gel system, ~1 μg aBWds1-L/lane could detect ~10 μg of Id + IgG. The solid-phase assay using hybridoma Id was more sensitive; 150 ng of ^{125}I-aBWds1-L reacted with 150 ng of serially diluted Id (cpm with an Id negative hybridoma averaged 175; cpm with 50 μg of BWds1 averaged 1,092; cpm with 150 ng of BWds1 averaged 338). The gel system was better than the solid-phase polyvinyl assay to detect Id plus bands in serum, in spite of its lower sensitivity, probably because the Id-bearing IgG was more concentrated in the gels.

Glomerular eluates. IgG was eluted from glomeruli of the second group of mice in groups A, B, and C (killed at 27 wk of age) by the method of Woodroffe and Wilson (15), except that sodium iodide (2 M, pH 9.0) was substituted for citrate buffer (pH 3.2). We have previously determined that sodium iodide extraction of glomeruli results in larger yields of IgG with anti-DNA activity than either acid elution or treatment with DNase. IgG and IgM were quantitated by immunodiffusion against rabbit anti-mouse IgG and IgM (Miles Laboratories, Inc.). Eluates were then studied by isoelectric focusing in 5% polyacrylamide gels containing urea, using the same methods as for serum. After focused bands were precipitated, they were overlaid with ^{125}I-dsDNA or with ^{125}I-aBWds1-L. All eluates were derived from pools of

kidneys from six to seven mice. Pooling is necessary to obtain enough IgG to visualize after focusing in gels.

RESULTS

Effects on deaths from nephritis. Deaths from nephritis, defined as animals dying with 2–4 + proteinuria and a blood urea nitrogen level > 20 mg/dl, are shown in Fig. 1. Deaths were delayed significantly in the group receiving BWdsl. By chi-square analysis (with Yates correction for small numbers), significantly fewer mice were dead in the BWdsl group compared with untreated controls at 28 ($P < 0.025$), 32 ($P < 0.05$), and 36 ($P < 0.05$) wk of age. Deaths in mice treated with LPC-1 were less frequent than in the untreated groups, but did not reach statistical significance at any time. The largest difference between the LPC-1 and untreated group was at 28 wk; $\Sigma\chi^2$ was 3.227, $0.1 < P > 0.05$. The differences between the LPC-1 and the BWdsl groups were significant at 32 and 36 wk ($P < 0.05$ at both times).

Proteinuria was also significantly delayed in the BWdsl-treated group, with a smaller proportion of those mice showing > 1 + proteinuria at 22 and 24 wk of age compared with the untreated mice ($P < 0.05$ at both times; data not shown). After 26 wk, there were no differences in the percentage of mice with proteinuria. At no time did the LPC-1 mice differ from the untreated group.

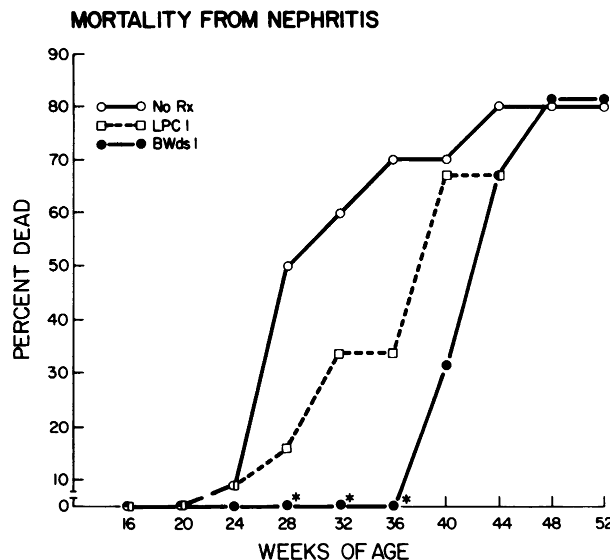

FIGURE 1 Mortality from nephritis. Percentage of mice in each group of 12 that died from nephritis is shown on the y-axis, weeks of age on the x-axis. Injections of monoclonal IgG were begun at 6 wk of age. Group A (●) received BWdsl; group B (□) received LPC1; group C (O) received no treatment. Asterisks indicate statistically significant differences between the untreated group and the BWdsl group (by Chi-square testing). Deaths were delayed ~12 wk by the administration of BWdsl. None of the differences between untreated and LPC-1 treated mice was significant.

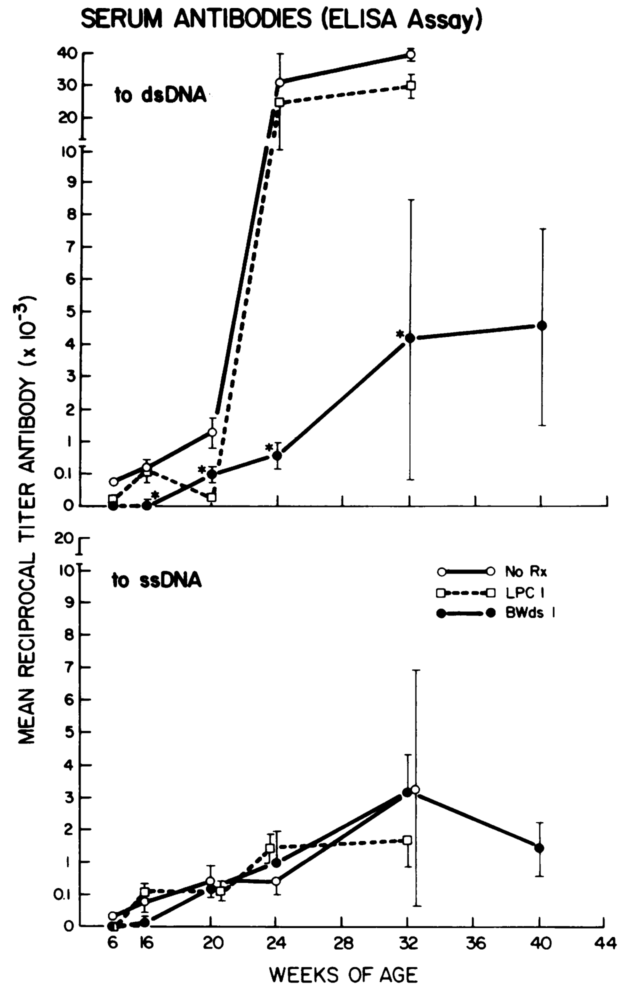

FIGURE 2 Circulating antibodies to DNA. Antibodies to calf thymus dsDNA and ssDNA were measured by ELISA assay (see text for details). The mean reciprocal titers of antibodies to dsDNA are shown in the upper panel; antibodies to ssDNA in the lower panel. Means are indicated by the points; vertical lines enclose 2 SEM. Asterisks indicate statistically significant differences (by Mann-Whitney U test). Antibodies to dsDNA were significantly reduced at 16, 20, 24, and 28 wk in the BWdsl group (●) compared to both LPC1 (□) and untreated (O) groups. Antibody titers to ssDNA were similar in all groups at all times.

Effects on circulating antibodies to DNA. Results of ELISA assays for serum antibodies to ds and ssDNA are shown in Fig. 2. Antibodies to dsDNA were present in high titers in untreated and LPC-1 treated mice by 24 wk of age. In contrast, anti-dsDNA titers were significantly lower in the BWdsl-treated group from 16 through 32 wk of age. Since only six untreated mice

were alive at 24–32 wk, differences between BWdsl and untreated mice were analyzed by the Mann-Whitney U test: P values for differences between the two groups were <0.025 at 16 wk, <0.005 at 20 wks, <0.025 at 24 and at 32 wk. The differences at each time were also significant at least at the $P = 0.05$ level by student's t test.

Antibodies to ssDNA, usually lower in titer than anti-dsDNA when measured by the ELISA technique in our colonies of NZB/NZW f_1 females, were not significantly different in the three therapeutic groups.

Tests for serum rheumatoid factors. Since disease in the mice could have been altered by the presence of anti-IgG induced by repeated injections of monoclonal IgG, we examined sera from six 24-wk-old mice in each group for the presence of rheumatoid factors. Results are shown in Table I. Mean disintegrations per minute of sera in each group were not higher than the negative control (normal mouse serum), nor did the groups differ significantly from each other. No individual serum was positive for rheumatoid factor.

DNA antibody spectrotypes. IgG bands binding dsDNA and contained in 24-wk-old sera were visualized after isoelectric focusing in vertical slab gels. A composite gel is shown in Fig. 3. BWdsl, focusing as four bands from pH 6.8–7.2, is shown in the center of the gel. Four sera from BWdsl-treated mice are in the left portion; three of the four did not contain detectable bands in the region from pH 6.2 to 7.2. Sera from all 12 mice in the BWds-1 group were studied; nine did not have detectable bands focusing at the same isoelectric point as BWdsl. In contrast, all six of the untreated mice alive at 24 wk had DNA binding bands in that region; two examples are shown in Fig. 3. Similarly, all 12 of the 24-wk-old LPC-1 treated mice had DNA binding bands from pH 6.2 to 7.2 (not shown in Fig. 3). These data suggested that administration of BWds-1 resulted in deletion of clonal IgG, perhaps of the same idiotype as BWdsl, or a cross-reacting idiotype.

TABLE I
Negative Tests for Rheumatoid Factor (Solid-Phase Assay)

Group	Mean dpm/well±SEM at two serum dilutions	
	1:6	1:96
MIgG Control	20,930	974
NMS Control	257	226
No Rx	136±8.6	170±2.3
BWds 1	135±9.4	154±11.8
LPC1	127±8.1	151±21.8

No individual mouse serum gave a positive result.

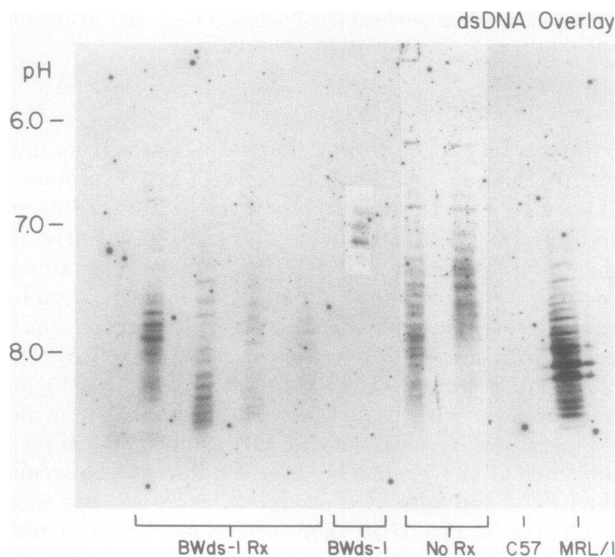

FIGURE 3 Deletion of IgG anti-DNA populations in BWds-1 treated mice. IgG from the sera of treated and untreated mice, from a normal C57Bl/6J mouse, and from an anti-DNA positive MRL/lpr mouse, as well as the IgG of BWdsl monoclonal anti-DNA, were concentrated and separated by isoelectric focusing in 5% polyacrylamide gels. After precipitation and fixation, the IgG bands were overlaid with ^{125}I-dsDNA. This is a composite gel showing BWdsl (four bands centering at pH 7.0) in the center. Sera from four 25-wk-old BWdsl-treated mice are shown in the left portion of the gel; sera from two untreated mice are shown to the right of BWdsl. All untreated mice had bands binding DNA from pH 6.2 to 9.0. Three of the four treated mice display no bands from pH 6.0 to 7.2. Among all the mice studied, six of the six untreated had bands focusing from 6.2 to 7.2, as did 12 of 12 LPC-1-treated mice; nine of 12 BWdsl-treated mice did not display bands in this region. The results suggest deletion of clonal IgG products with isoelectric points similar to that of BWdsl.

Anti-idiotypic antibodies. Initially, we tested for anti-Id by incubating whole serum with ^{125}I-BWdsl in a solid-phase assay. Iodination of the clonal IgG probably did not significantly alter its antigen binding capacity, since 1 μg of the IgG bound 20 ng of dsDNA before iodination, and 18 ng after iodination, as measured in the Farr assay. ^{125}I-labeled normal mouse IgG and ^{125}I-LPC-1 served as controls. None of the sera from BWdsl-treated mice, tested at 16, 24, and 30 wk of age, bound iodinated BWdsl at quantities higher than the binding of either control IgG. In fact, counts per minute bound to the microtiter plates were low (averaging 110) for sera from mice in all three treatment groups.

Therefore, sera were incubated with urea, and IgG proteins were separated and concentrated by isoelectric focusing in vertical slab gels, then overlaid with ^{125}I-BWdsl. Multiple bands that bound BWdsl were

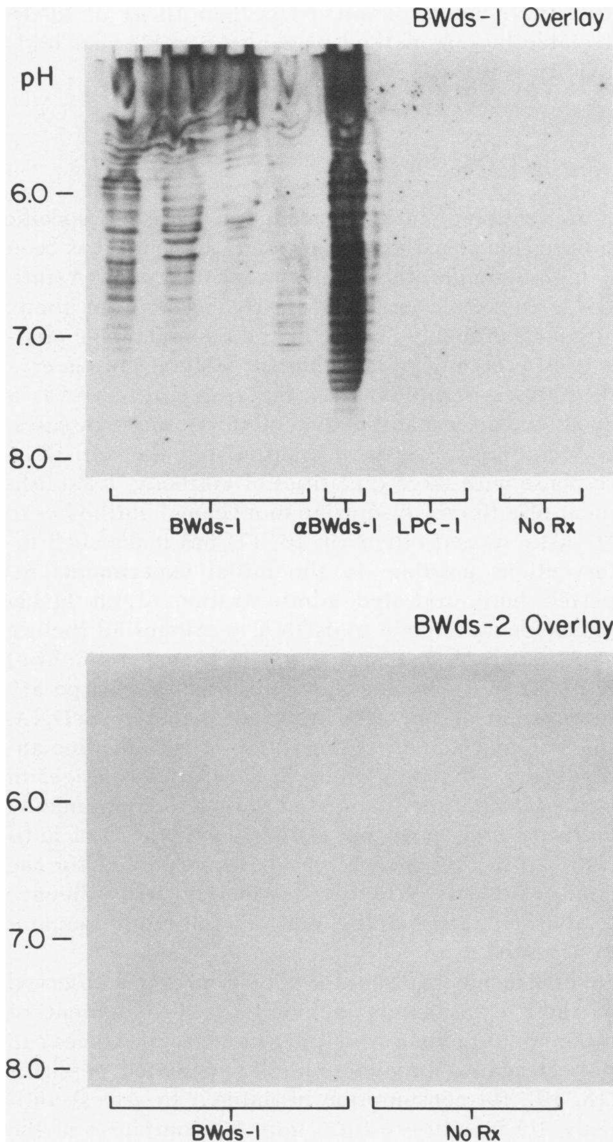

FIGURE 4 Evidence for anti-idiotypic antibodies to BWds1. Sera from BWds1-treated mice and from the LPC-1 and untreated control groups were studied by isoelectric focusing as in Fig. 3. A heterologous mouse antiserum to BWds1, aBWds1, is displayed in the center of the gel. The IgG bands were overlaid with ^{125}I-BWds1 (upper panel) or with another monoclonal anti-DNA of the same allotype, BWds2 (lower panel). All sera from BWds1-treated mice bound BWds1 but not BWds2; the same was true for aBWds1 (not shown in the lower panel). Sera from LPC-1 and untreated mice did not contain any bands capable of binding either BWds1 or BWds2 (LPC-1 sera not shown in lower panel). Fab portions of BWds1 gave results identical to those shown in the upper panel. These data strongly suggest the presence of anti-idiotypic antibodies to BWds1 in sera of mice inoculated with that idiotype.

detected in sera from BWds1-treated mice (12 of 12 at 24 to 30 wk of age) but not in any of the sera from LPC-1 treated or untreated mice (0 of 6 untreated, 0 of 8 LPC-1 treated). A portion of these results are shown in Fig. 4. Heterologous anti-Id to BWds1 raised in LPJ mice (aBWds1) are shown in the center of the upper gel as a positive control.

As shown in the lower panel of Fig. 4, the sera did not bind ^{125}I-BWds2, another IgG2a kappa monoclonal antibody derived from a different NZB/NZW f_1 spleen fusion from BWds1. The Fab portion of BWds1 was also iodinated and bound to serum bands from all of the BWds1-treated mice, giving a pattern identical to that of the intact BWds1 molecules.

The presence of anti-Id against BWds1 was further confirmed by the growth of one clone yielding IgG antibody to Id (Fab portions reacting with BWds1 but not BWds2) from a hybridoma derived from one spleen from a 25-wk-old BWds1-treated NZB/NZW f_1 female. This clone was designated aBWds1-B1.

Alteration of the spectrotypes of anti-Id occurred with time and are illustrated in Fig. 5. An isoelectric focusing gel containing serial serum specimens from three BWds-1 treated mice (at 12, 20, and 30 wk of age) is shown. After overlay with ^{125}I-BWds1, multiple bands appeared in all specimens, with more alkaline bands appearing in later specimens. However, in all 30-wk specimens, deletion of bands present in earlier specimens was evident, suggesting drop-out of certain clonal products with time.

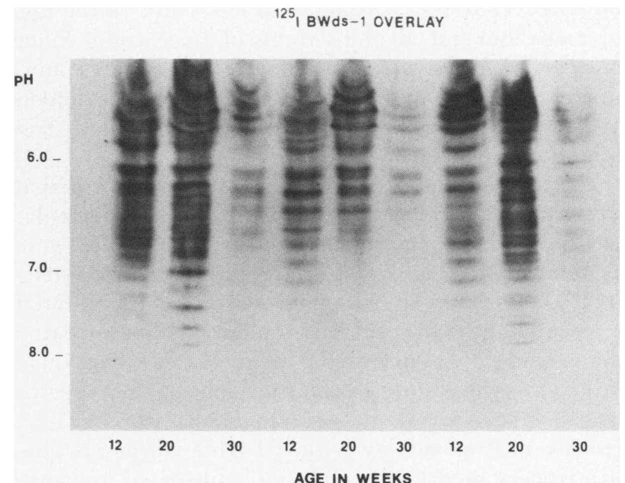

FIGURE 5 Alteration of spectrotypes of anti-Id with time. Sera from three BWds1-treated mice were studied by isoelectric focusing and ^{125}I-BWds1 overlay at 12, 20, and 30 wk of age. With time, some IgG bands disappear and cationic bands (pI 7.8–8.8) appear. There was probably deletion of some anti-Id clonal products, as there was for Id (see Fig. 4).

Additional gels containing focused IgG bands from the BWds1-treated and control mice shown in Fig. 4 were overlaid with cold DNA (1.44 mg per gel), incubated at 37°C for 1 h, then overnight at 4°C. They were then incubated with ^{125}I-BWds1. The same bands bound Id as in the experiments without DNA; the gel was identical to that shown in the upper panel of Fig. 4. Therefore, we could not demonstrate interference with the Id-anti-Id reaction by DNA antigen.

Frequent occurrence of the BWds1 idiotype. Eight monoclonal antibodies to DNA (derived from eight NZB/NZW f_1 female spleens) and individual sera from 15 untreated NZB/NZW females were subjected to isoelectric focusing, then overlaid with two radioiodinated anti-Id preparations. Those preparations included (*a*) one LPJ heterologous antiserum and (*b*) one LPJ-mouse-derived monoclonal anti-Id (aBWds1-L). The monoclonal preparation contained 150 μg/ml of IgG anti-Id; the concentration of anti-Id in the heterologous antiserum was not determined, but was probably much lower.

None of the monoclonal anti-DNA antibodies and none of the protein bands from serum bound the heterologous anti-Id. However, four of the eight hybridomas (from eight different mice), and seven of 15 sera had bands that bound aBWds1-L. All positive sera contained Id-positive bands at pH 6.2–7.2: a few also had Id-positive bands from pH 7.5–8.5. Overlay of similar gels with ^{125}I-dsDNA showed binding of DNA by the same anti-Id-positive bands. These data suggested that BWds1 bears an Id that occurs frequently on NZB/NZW IgG antibodies to DNA and on the IgG of many but not all individuals of that strain. Since our assay is relatively insensitive for sera and glomerular eluates focused in gels (1 μg of anti-Id/lane detects 10 μg of Id), the Id may be present even more frequently than we have estimated.

Glomerular eluates. BWds1-treated mice, killed at 27 wk of age, had lower quantities of IgG in glomerular eluates than did the untreated controls. Values were as follows: 250 μg IgG/g of renal tissue in BWds1-treated mice; 500 μg IgG/g of renal tissue in untreated mice; 500 μg IgG/g of renal tissue in myeloma-treated mice. No IgM was detected. When eluates (80 μg IgG/ml) were subjected to isoelectric focusing and precipitated with Na_2SO_4, no protein bands were visible. However, after overlay with ^{125}I-DNA, three alkaline bands were seen in the eluates of untreated and myeloma-treated mice; no bands were detectable in BWds1-treated mice. After overlay with anti-Id, ^{125}I-aBWds1-L, none of the bands was visible. Therefore, we were not able to detect Id in the alkaline bands derived from pools of kidney tissue from six to seven mice in each of the two control groups; it is possible that the eluates contained less than 10 μg of Id detectable by our method. It is also possible that high-avidity antibodies were not eluted.

DISCUSSION

Amelioration of antibody-mediated disease by specific suppression of pathogenic immune responses has been a highly sought therapeutic goal. Since DNA-anti-DNA immune complexes clearly account for some, although probably not all, of the tissue damage characteristic of murine and human systemic lupus erythematosus, suppression of this response serves as a good model for manipulation of autoimmune diseases.

Accordingly, we have manipulated the anti-DNA response with large quantities of antibody. Establishment of a library of murine monoclonal antibodies to DNA by us and others (9, 16, 17) has made such interventions possible. In the initial experiments reported here, repeated administration of an IgG2a monoclonal antibody to dsDNA to premorbid (before appearance of circulating anti-DNA or proteinuria) NZB/NZW f_1 female mice resulted in temporary suppression of (*a*) circulating antibodies to dsDNA, (*b*) proteinuria, and (*c*) deposition of IgG alkaline antibodies to DNA in glomeruli. Survival was longer in treated mice. Anti-Id directed against the immunizing antibody may have suppressed the synthesis of anti-DNA antibodies, which may have accounted for the clinical benefits. Whether this method will influence established disease in the morbid adult mouse remains to be tested.

Possible mechanisms for the suppression observed in these experiments included (*a*) development of rheumatoid factors, which in some circumstances can protect against immune complex-mediated nephritis (18, 19), (*b*) consumption of antigen by excess antibody, (*c*) inability to form immune complexes of the correct pathogenic size or configuration in a state of antibody excess, (*d*) suppression of B cell antibody production by the negative feedback mechanism of antibody excess, (*e*) suppression of B cell antibody synthesis by anti-Id cross-reacting with multiple different antibodies to DNA, and (*f*) suppression of B cell antibody synthesis by activation of Id-bearing suppressor T cells or inactivation of Id-bearing helper cells. Many of these mechanisms may be interrelated, and it is likely that several were operational simultaneously.

Circulating rheumatoid factors were not detectable in any of the mice studied. Although anti-IgG antibodies are characteristically present in another murine systemic lupus erythematosus strain, MRL/lpr (20), they did not occur either spontaneously or after re-

peated injections of BWds1 or the LPC-1 myeloma IgG in our mice. Thus, our mice were stimulated to make anti-Fab antibodies but not anti-Fc; the reason for this is unknown although rheumatoid factors are not common in the NZB/NZW strain (20).

It is certainly possible that the large quantities of anti-DNA inoculated into experimental mice consumed DNA antigen, so that ultimately none was available for formation of pathogenic immune complexes. We did not test this hypothesis.

We can address the possible suppression of B cell synthesis of antibodies to DNA by anti-Id. Anti-Id of the IgG class was detectable in the sera of BWds1-treated animals after three inoculations of Id and was detected in all treated animals. Anti-Id was not demonstrable when whole serum was reacted with Id, but only after isoelectric focusing of the IgG. This is probably accounted for by the greater concentration of Id-bearing IgG after focusing, although separation of Id-anti-Id by urea treatment in the trough and gel, as well as elimination of interfering substances by focusing, may also be important. Evidence for the reaction representing Id-anti-Id interactions included the ability of Fab fragments of Id to react with the BWds1 sera as well as the whole IgG molecule, and, most importantly, the failure of the serum bands to react with BWds2, an IgG monoclonal anti-DNA with the same allotype as BWds1. Our ability to immortalize one monoclonal anti-Id from the spleen of a BWds1-treated mouse further confirms the capacity of the treated mice to produce anti-Id. It is interesting that the Id-anti-Id interaction in polyacrylamide gels could not be blocked by prior incubation with DNA. It is possible that the Id binds anti-Id at a region other than the antigen-combining site.

Anti-BWds1L proved to be cross-reactive with other NZB/NZW-derived antibodies to DNA, including four of eight hybridoma-derived IgG antibodies, and seven of 15 NZB/NZW sera. Commonly shared or widely cross-reactive Id were characteristic of anti-DNA monoclonal antibodies derived from NZB/NZW mice by Marion et al. (22). Similarly, Rauch and colleagues (23) derived a heterologous anti-Id that cross-reacted with 40–60% of serum antibodies to DNA in MRL/lpr mice, and with a majority of hybridoma-derived monoclonal antibodies from that strain. The anti-Id in their system, however, did compete with DNA for binding of Id. The Id was not confined to IgG or to antibodies specific for DNA, suggesting that our technique of suppressing anti-DNA with anti-Id may suppress more than one antibody system.

The presence of widely cross-reactive anti-Id in all of the BWds1-treated mice may have played a major role in temporarily suppressing synthesis of all antibodies to DNA. As postulated by Jerne (24), interactions between Id and anti-Id are probably essential in controlling the immune response. Both B and T cells are susceptible to ultimate "up" or "down" regulation by interaction of cells and anti-Id (21, 25–27). There is some evidence that humans with systemic lupus erythematosus reduce their anti-DNA responses and enter clinical remissions when anti-Id reactive with anti-DNA appears in their serum (28). We therefore suspect that the hyperimmunization with a frequently occurring Id resulted in stimulation of anti-Id, which subsequently suppressed the anti-DNA response by direct action upon B and T cells. Whether deletion of anti-Id clones within the spectrotypes of anti-Id that occurred with time accounted for the loss of DNA tolerance remains to be examined. Since we now have large quantities of monoclonal Id and of anti-Id derived both from LPJ and from NZB/NZW mice, we can test these hypotheses in vitro. These experiments are in progress and should also provide answers to the question regarding the role of Id+ suppressor T cells in this system.

A comment regarding the role of electrical charge and pathogenicity of different antibodies to DNA should be made. We have previously reported that only IgG antibodies to DNA with alkaline isoelectric points can be isolated from NZB/NZW glomeruli (3), and that cationic charge may be necessary for localization of large quantities of immune complexes to glomerular basement membranes. This hypothesis is consistent with the data of Gallo et al. (29). BWds1 has a neutral isoelectric point averaging 7.0; neutral antibodies to DNA were deleted from the sera of mice protected from nephritis in our experiments. However, although cationic antibodies were present in the circulation of BWds1-treated mice, their quantities were greatly reduced, and they could not be found in renal eluates from 25-wk-old treated mice, whereas they were present in untreated and myeloma-treated mice. Therefore, it is probably the quantitative and not the qualitative change in antibody spectrotype that accounted for protection from nephritis.

It is possible that treatment with LPC-1 prolonged survival somewhat, even though differences did not reach statistical significance. LPC-1 does have antibody reactivity with phosphorylcholine, and it is conceivable that it may be able to bind to certain cell membranes and thus alter cell function. Furthermore, some monoclonal IgG2a proteins can interact with Fc receptors in murine mast cells and activate those cells, thus potentially modifying the immune response (30). However, no effect of LPC-1 on proteinuria, glomerular IgG deposition, or quantity of circulating antibodies to DNA was found, so if it influenced mouse

survival, its effects were probably quite different from those of BWdsl.

In summary, we have developed a novel method of reducing the humoral immune response to dsDNA that characterizes NZB/NZW murine lupus. Antibody and disease suppression were associated with the appearance of anti-Id, which may have played a role in the reduction of DNA antibody synthesis. Experiments to elucidate the mechanism of antibody control and to prolong the period of "tolerance" are in progress.

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