Treatment of Lupus Nephritis in Adult (NZB + NZW)F_1 Mice by Cortisone-Facilitated Tolerance to Nucleic Acid Antigens

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A B S T R A C T Adult female (NZB × NZW)F_1 mice were treated with cortisone, cortisone with tolerogen (isologous NZB IgG-nucleosides conjugates) or cortisone with isologous IgG free of nucleosides. Other treatments also included tolerogen or isologous IgG alone, and cortisone together with denatured DNA. All untreated mice died by 10 mo of age. Cortisone prolonged the survival rate. This effect was further improved by combined treatment of cortisone and tolerogen. Prolonged survival was accompanied by a decrease in proteinuria. Other treatments failed to influence either survival or proteinuria. Although cortisone did not prevent the appearance of antibody to denatured DNA, cortisone and tolerogen suppressed them in most of the animals. Preexisting antibody to denatured DNA was reduced by cortisone and cortisone and tolerogen, but not by cortisone and IgG. In contrast, antibody to native DNA bore no relationship to therapy. Animals living beyond 1 yr of age, regardless of the treatment, fall into three histopathological categories: (a) severe nephritis, as in untreated animals, (b) moderate nephritis (with absence of severe alteration of the glomerular basement membrane, i.e. the histological counterpart of prolonged survival), (c) minimal nephritis. In a small number of animals treated with cortisone or cortisone and IgG and in 6/20 animals treated with cortisone and tolerogen, minimal lesions as judged by light, fluorescent, and electron microscopy were found. These last mice were in good health at 15–16 mo of

age, twice the life-span of untreated mice. In conclusion, these data suggest that tolerance to nucleic acid antigens facilitated by cortisone offers a promising new approach to treat established murine lupus nephritis.

INTRODUCTION

(NZB × NZW)F_1 (hereafter referred to as BWF$_1$)[1] mice develop an immunologic disorder that closely resembles systemic lupus erythematosus (SLE) in man (1, 2). In the female BWF$_1$ mouse, the disease manifests itself by 6 mo of age, with persistent proteinuria and circulating antibodies to nucleic acids. The primary cause of death in these animals is renal failure due to progressive immune complex-mediated glomerulonephritis (3, 4). Nucleic acid antigens, such as single-stranded DNA(D-DNA), appear to play an important role in the development of the renal lesion (3).

Prevention of murine SLE by immunological means has been attempted by a method in which the production of anti-D-DNA antibodies was reduced by the neonatal administration of nucleosides conjugated to isologous IgG (5). Although this form of hapten-specific carrier-induced immunologic tolerance may prevent SLE, of greater importance is the possibility that similar methods might be used to treat adult animals exhibiting signs of clinical disease.

[1] *Abbreviations used in this paper*: ANAB, antinuclear antibody; BWF$_1$, NZB × NZW F_1 hybrid mice; (C), cortisone; D-DNA, denatured DNA; GBM, glomerular basement membrane; N-DNA, native DNA; SLE, systemic lupus erythematosus; (T), tolerogen.

Recently, the facilitation of tolerance induction in vitro (6) and in vivo[2] by high doses of glucocorticosteroids has been reported. This phenomenon has been found to be the result of a cortisone-dependent shift in the amount of antigen required for the induction of both tolerance and immunity (6). In mice injected 24 h previously with hydrocortisone, an antigen concentration considered optimally immunogenic for normal animals was found to be tolerogenic, whereas up to 500-fold less antigen proved to be optimally immunogenic.[2] In view of the potential therapeutic significance of this observation, we applied it to the treatment of the adult BWF_1 mice with clinical signs of lupus nephritis as indicated by the presence of anti-DNA antibody and proteinuria.

METHODS

Animals. The animals used in these experiments were the first generation hybrid female offspring derived from a closed colony of NZB × NZW mice (BWF_1). The colony was originally obtained from England and was bred by the Health Services Animal Center, University of Alberta.

Preparation of carriers and tolerogen. NZB IgG was prepared from NZB serum by starch-block electrophoresis as previously described (7). Nucleosides (A-adenosine, G-guanosine, C-cytidine, T-ribothymidine, purchased from Sigma Chemical Co., St. Louis, Mo.) were conjugated to NZB IgG by the method of Erlanger and Beiser as previously described (5). Tetranucleoside tolerogen preparations ($AGCT_{25}$-IgG) and ($AGCT_{15}$-IgG) were used in the experiments described. (Subscript numbers indicate the molar ratio of hapten to carrier.)

Cortisone acetate. Cortisone acetate in carbonylmethyl cellulose was purchased from Merck Sharp & Dohme, Ltd., Kirland, Quebec. Mice were injected subcutaneously with 10 mg/30 g 24 h before the tolerogen.

Denatured DNA (D-DNA). Heat-denatured calf thymus DNA used for treatment was a gift from Dr. R. Morgan, Department of Biochemistry, University of Alberta.

Detection of antibody to DNA. The radioimmunoassay for anti-DNA antibody was a modification of the technique described by Lewis et al. (8) Briefly, ^{14}C-labeled native DNA (N-DNA; New England Nuclear, Boston Mass.) with less than 6% single-strand DNA, or heat-denatured deoxyribonucleic acid (D-DNA) was incubated for 20 min with heat-inactivated test sera, and the reaction mixture passed through a glass fiber filter under suction. Free radiolabeled antigen passed through the filter, whereas antigen complexed to antibody was retained. The ratio of radiolabeled antigen retained by the filter as compared to the total amount of radioactivity in the reaction mixture was expressed as the binding percentage for N-DNA or D-DNA. Upper limits of normal binding are 10% for D-DNA and 20% for N-DNA; these values are 2 SD above the mean background values.

Antinuclear antibody (ANAB). The ANAB test was conducted by incubating whole test serum with 4-μm frozen sections of perinatal rat liver for 30 min at 20°C in a humid chamber, washing the sections twice with buffered saline, and reincubating them for 20 min with the globulin fraction of a fluorescein-conjugated rabbit antiserum to mouse IgG. After thorough washing, the specimen was examined

for nuclear fluorescence by ultraviolet microscopy. Positive reactions were recorded, the staining pattern described, and serial twofold dilutions of positive sera were prepared to determine the titer of ANAB present in the specimen. Positive titers ranged from 1:4 to 1:512.

Antemortem examination. (a) Physical examination: All mice in these experiments were examined visually on a daily basis for signs of illness. Whole body weights were recorded monthly and when judged moribund, the animals were sacrificed. (b) Proteinuria: Dip stick urinanalysis (Difco Laboratories, Detroit, Mich.) was performed once a month until significant proteinuria was observed (300 mg/ml or above). Thereafter urinalysis was performed weekly. (c) Blood urea nitrogen: Test strip examination for levels of blood urea nitrogen was conducted from tail vein blood once monthly until signs of azotemia developed (20 mg/100 ml or above). Thereafter blood urea nitrogen determinations were conducted weekly.

Pathological examination. ·(a) Postmortem examination: A complete necropsy (central nervous system excluded) was performed on all control and test animals used in these experiments. Moribund animals were selected for sacrifice by exsanguination after ether anesthesia. Histopathological examination for all major organ systems was routinely carried out. (b) Fluorescent microscopy: Kidneys obtained either from fresh necropsy material or by unilateral nephrectomy were transversely sectioned into 2-mm blocks, snap frozen in a slurry of dry ice and acetone, and stored at −70°C before processing. 2-μm frozen sections were prepared and stained with fluorescein-conjugated heterologous antisera to murine albumin, fibrinogen, beta 1C globulin, IgG, and polyvalent anti-IgG, IgA, and IgM. The degree, location, character, distribution, and morphology of fluorescent staining were recorded on a standardized grading chart, and representative lesions were photographed for inclusion into each animal's permanent record. The number of glomeruli examined in each section ranged between 75–125. (c) Electron microscopy: Tissues were prepared for electron microscopy as previously described (9). A minimum of 40 glomeruli were studied from each sample, and a uniform grading system was utilized to record data. Thus, data obtained from lesions in each group of experimental mice were compared and evaluated for qualitative and quantitative differences. (d) Kidney elution studies: Acid glycine buffer, pH 3.0, was used to dissociate antibody from immune complexes lodged in affected glomeruli as described by Koffler et al. (10).

After the elution procedure, the immunoglobulin content was authenticated by immunoelectrophoresis, and the eluates were adjusted to a standard protein concentration (0.4 mg/ml). Eluates prepared in this fashion were then tested for immunologic reactivity to nucleic acid and viral antigens using the radioimmunoassays described by Lewis (8) and Scolnick et al. (11).

Experimental protocol. Serial studies of untreated female BWF_1 mice from 3 mo of age until death formed the basis of control data related to the current experiments. Adult female mice 6 mo of age were divided into the following groups: untreated, treated with cortisone alone (C), cortisone and tolerogen (C + T), tolerogen alone (T), IgG alone (IgG), cortisone and IgG (C + IgG), and cortisone and D-DNA (C-DNA). The treatment schedule is summarized in Table I.

RESULTS

Survival. Untreated control female BWF_1 mice in our colony predictably died in renal failure between 6 and 7 mo of age. Only occasional animals lived

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[2] Diener, E. Unpublished observations.

TABLE I
Treatment Schedule

Group	First treatment at 6 mo of age	Treatment 24 h later	Treatment 3 wk later	Maintenance treatment
I	None			
II	Cortisone 10 mg s. cut		Cortisone 10 mg s. cut	
III	Cortisone 10 mg s. cut	Tolerogen 1 mg i.v.	Cortisone 24 h 10 mg s. cut	50 µg of tolerogen i.p. every 2 wk
IV		Tolerogen 1 mg i.v.		50 µg of tolerogen i.p. every 2 wk
V		NZBIgG 1 mg i.v.		50 µg NZBIgG i.p. every 2 wk
VI	Cortisone 10 mg s. cut	NZBIgG 1 mg i.v.	Cortisone 24 h 10 mg s. cut	50 µg of NZBIgG i.p. every 2 wk
VII	Cortisone 10 mg s. cut	D-DNA 1 mg i.v.	Cortisone 24 h 10 mg s. cut	50 µg of D-DNA i.p. every 2 wk

Three separate experiments were carried out to evaluate the effects of cortisone (C) as compared to cortisone in combination with the tolerogen (C + T) on the survival rate of female BWF$_1$ mice. Although some variability occurred between experimental groups, the overall effect of treatment with cortisone alone was to extend the 50% survival rate to at least 12 mo of age, confirming the results of others (12). However, the beneficial effect of cortisone was further extended when it was combined with tolerogen. Cortisone and IgG also appeared to have some beneficial effect, whereas tolerogen alone, as well as cortisone and D-DNA, failed to influence the rate of survival. The efficacy of the combined (C + T) treatment schedule is clearly shown by the survival of one-half (46%) of the mice in this group to 15 mo of age, whereas all but one animal treated with cortisone alone were dead at 15 mo.

Proteinuria. Serial urinalysis was used to monitor the development of glomerulonephritis in these experiments. At the onset of the experiment <10% of all animals had proteinuria above 300 mg/100 ml. As illustrated in Table III, 80% of the untreated control mice had significant (>300 mg/100 ml) proteinuria by 7 mo of age. Urinary protein loss at this level or

to 9 mo of age and all mice were dead before 1 yr (Table II). Terminally, all of these animals had antibodies to D-DNA, high proteinuria (>300 mg/ml), an elevated blood urea nitrogen (>40 mg/ml), and severe immune complex-mediated glomerulonephritis.

TABLE II
Survival Rate

Group	Treatment	6	7	8	9	10	11	12	13	14	15	16
I	None	10/13	5/13	1/13	1/13	0/13						
II	Cortisone											
	1	8/8	8/8	8/8	8/8	7/8	6/8	3/8	0/8			
	2	10/10	7/10	7/10	6/10	6/10	5/10	4/10	1/10	0/10		
	3	9/9	9/9	9/9	9/9	8/9	8/9	6/9	4/9	3/9	1/9	
		27/27	24/27	24/27	23/27	21/27	20/27	17/27	5/27*	3/27	1/27	0/27
III	Cortisone and tolerogen											
	1	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7	5/7
	2	10/10	9/10	7/10	7/10	7/10	7/10	7/10	3/10	0/10	—	—
	3	9/9	9/9	9/9	7/9	7/9	5/9	5/9	5/9	5/9	5/9	5/9
		26/26	25/26	23/26	21/26	21/26	19/26	19/26	15/26*	12/26	12/26	10/26
IV	Tolerogen	8/8	0/8									
V	IgG	6/6	6/6	5/6	4/6	3/6	3/6	3/6	3/6	0/6		
VI	Cortisone and IgG	8/8	8/8	8/8	8/8	7/8	7/8	6/8	6/8	4/8	2/8	
VII	Cortisone and D-DNA	6/8	5/8	5/8	4/8	3/8	2/8	0/8				

* Significantly different from control group I ($P < 0.01$ according to χ^2-test).

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TABLE III
Proteinuria

Group	No. BWF1 mice	Treatment	7	9	12	15
I	13	None	8/10*	1/1	0/0	0/0
II	27	Cortisone	4/27	10/23	9/13	1/1
III	26	Cortisone and tolerogen	1/26	1/24	3/19	3/12
V	6	IgG	6/6	2/4	2/3	—
VI	8	Cortisone and IgG	0/8	0/8	1/6	2/2
VII	8	Cortisone and D-DNA	2/8	4/4		

Header note: columns 7, 9, 12, 15 are under "Age, mo".

* Number of mice with significant proteinuria >300 mg/100 ml per number of survivors.
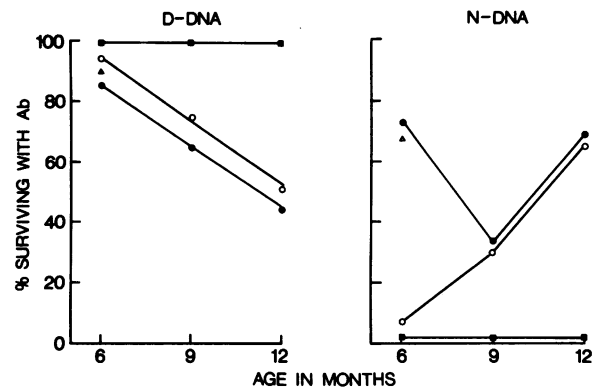

FIGURE 1 Results of antibody to DNA in animals with antibody to D-DNA. Experiments 1 and 3. Animals treated with cortisone and IgG (■), cortisone (○) and cortisone and tolerogen (●) between 6 and 9 mo. $P = 0.32$ for cortisone-treated and $P < 0.025$ for cortisone plus tolerogen-treated groups according to χ^2 test.

higher regularly preceded the development of azotemia and death.

Treatment with cortisone delayed the onset of proteinuria to the point that by 9 mo of age, only 55% of these animals suffered from significant urinary protein loss. However, this effect was short lived and by 12 mo of age, 70% of the surviving animals in this group had significant proteinuria. As was seen in survival rates, the combination of tolerogen and cortisone was the most effective treatment in preventing or retarding the development of proteinuria. At 9 mo of age, less than 10% of the animals in the (C + T) treatment group had proteinuria above 300 mg/ml and at 15 mo of age, 75% of the living animals still failed to exhibit significant loss of urinary protein. Treatment with cortisone and IgG was also effective in retarding the development of proteinuria in animals surviving to 12 mo of age. None of the other treatment schedules prevented the early development of significant proteinuria.

Serology. Approximately 90% of the animals selected for treatment with cortisone (C), cortisone + tolerogen (C + T), or cortisone + IgG (C + IgG) had antibody to D-DNA before the onset of the experiment (groups 1 and 3 of Table II). Once treatment was initiated, the percentage of serologically positive animals decreased in both the (C) and (C + T) groups, an event which paralleled their prolonged survival. The observed decrease in percentage of seropositive animals in these two groups, however, was only mild and at 12 mo of age, ≅50% of all animals receiving treatment were making anti-D-DNA antibody. Treatment with cortisone and IgG had no beneficial effect between 6 and 12 mo of age. The presence or absence of anti-N-DNA antibody bore no relation-

ship to the treatment groups in these experiments (Fig. 1).

To assess the effects of treatment on mice not yet producing antibody to D-DNA, 20 animals that lacked antibody to D-DNA at 5.5 mo of age, but that had significant binding values for antibody to N-DNA were selected (group 2 of Table II). The institution of treatment with cortisone (10 animals) or cortisone and tolerogen in 10 other animals elicited a difference in their serological profile after therapy. Cortisone alone had no influence on the development of antibody to D-DNA and by 9 mo of age, five of six of the surviving animals had antibody to this antigen. By contrast, only two of seven of the animals treated with cortisone and tolerogen had developed antibody to D-DNA by this time (Fig. 2).

The beneficial effect of cortisone and tolerogen was most evident at 12 mo of age, at which time
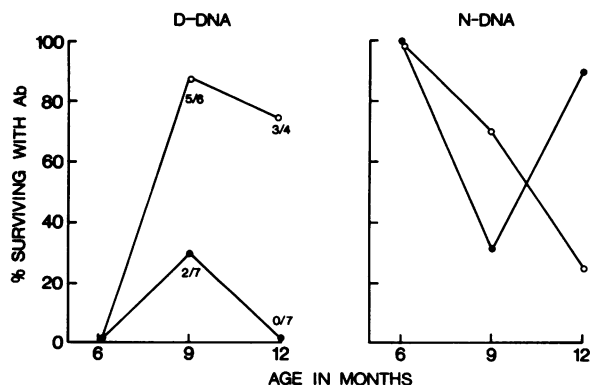

FIGURE 2 Results of antibody to DNA in animals with antibody to N-DNA. Experiment 2. Animals treated with cortisone (○) and with cortisone and tolerogen (●).
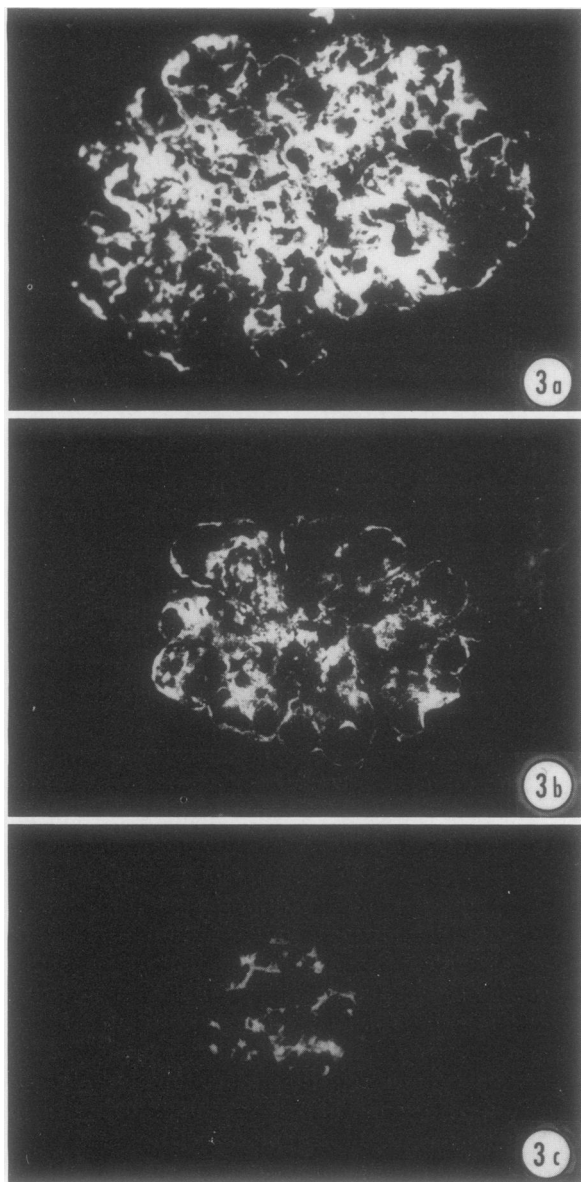
FIGURE 3 (a) Severe glomerulonephritis. Immunofluorescent lesion of an untreated 6-mo-old control mouse stained with fluorescein-conjugated rabbit anti-mouse IgG. Discrete small- to medium-sized deposits of IgG are present along capillary basement membranes and large, coarse granular deposits occupy the mesangium and thickened glomerular capillary walls. A similar staining pattern is observed when serial sections are stained for beta 1C globulin. (b) Mild to moderate glomerulonephritis. Discrete focal deposits of IgG and beta 1C globulin are present along capillary basement membranes. Mild staining of the mesangium is present. Capillary walls are generally not thickened, nor are their adhesions of glomerular tufts to Bowman's capsule. This photograph is representative of the moderate lesion found in 15-mo-old BWF₁ mice successfully treated with cortisone and tolerogen. (c) Normal kidney. Fluorescent staining pattern of a glomerulus from a 16-mo-old BWF₁ mouse successfully treated with cortisone and tolerogen. An arboriz-

none of the seven surviving animals in this group (7/10) had anti-D-DNA antibody. In contrast, three of four of the survivors in the group treated with cortisone alone were seropositive for anti-D-DNA antibody. As observed in the earlier experiment, treatment with cortisone or cortisone and tolerogen had no observable effect on the development of ANAB or anti-N-DNA antibody, suggesting that the suppression of antibody to D-DNA by tolerogen administration was specific.

Pathology

Untreated control mice. The lesions observed in this group were consistent with those previously reported to be characteristic for BWF₁ mice (13–17). By light microscope examination, severe membrano-proliferative glomerulonephritis was uniformly present at 6 mo of age. In addition to the usual intra-glomerular membranous and proliferative changes associated with this form of nephritis, periglomerular and periarterial lymphocytic and plasma cell infiltrates were a consistent and prominent finding. Immunofluorescent studies regularly demonstrated focal discreet deposits of immunoglobulins and beta 1C globulin along glomerular capillary basement membranes and in the walls of some thickened glomerular capillary loops. Large coarse granular deposits of stain were also present in the mesangium and at sites of adherence of glomerular tufts to Bowman's capsule (Fig. 3 a). Albumin and fibrinogen were generally not demonstrable in the glomerular lesion.

The ultrastructural lesion was characterized by (*a*) irregular thickening of the glomerular capillary basement membrane (GBM) with accumulation of electron-dense deposits within the membrane and in subepithelial and subendothelial locations; (*b*) hypertrophy of visceral epithelial cells and fusion of podocytes over the GBM; (*c*) extensive proliferation of mesangial and endothelial cells, which obliterated capillary lumina and distorted the normal glomerular cytoarchitecture. Marked electron-dense deposits were constant in the mesangium both in the GBM-like substance and in the mesangial cells. Immature C-type virus particles were observed budding from visceral and parietal epithelial cells within glomeruli, and mature virus particles were seen in the infoldings of the plasma membrane of convoluted tubules (18).

Experimental treatment groups. Mice subjected to the various treatment schedules listed in Table I reflected two distinct patterns of disease regardless of

ing pattern of mesangial stain is present. Capillary basement membranes lack deposits of either IgG or beta 1C globulin. The staining pattern of the mesangium is characteristic of aging changes observed in mice of other strains and does not reflect immunologic injury to the glomerular capillaries.

the therapeutic regimen. (a) The first was comprised of sick animals with clinical, chemical, and pathological evidence of severe glomerulonephritis, irrespective of their age at the time of sacrifice. The lesions in these mice were identical to those observed in the untreated mice (Fig. 4 a). (b) The second pattern contained those animals that appeared clinically normal and exhibited only intermittent signs of illness and minor laboratory abnormalities before the time of sacrifice. Mice in this category could be further divided into two groups: those with mild to moderate histopathologic lesions and definite immunofluorescent evidence of diffuse immune complex-mediated glomerulonephritis (Fig. 3 b); and those animals with no significant histopathologic lesions. In the first case, ultrastructural analysis revealed that the moderate histopathologic lesion was characterized by mild, very discrete focal changes in the basement membrane and marked proliferation of endogenous glomerular cells. The GBM appeared thin except for very restricted segments of irregular thickening associated with electron-dense deposits. Epithelial cell foot processes were normal. The major and outstanding abnormality was engorgement of capillary lumina by swollen, distended, and proliferating endothelial and mesangial cells (Fig. 4 b). In addition, electron-dense deposits were regularly present in the mesangium, and periglomerular lymphoid cellular infiltrates were common. The number of treated mice with moderate lesions were 5 (C), 8 (C + T), and 2 (C + IgG).

In the second case, no glomerular lesions were observed by light microscopy, and only minor segmental fluorescent lesions were present in occasional glomeruli (focal glomerulitis). Ultrastructurally, the GBM and epithelial cell foot processes were normal, and most capillary lumina were open (Fig. 5 a). Mild mesangial cell proliferation but marked electron-dense deposits in the mesangial matrix were the only commonly encountered abnormalities in animals of this group (Fig. 5 b).

In all experimental groups, C-type virus particles were observed in approximately the same numbers and in the same locations as described for control mice.

Analysis of the combined pathological findings in 20 mice treated with cortisone and tolerogen revealed that six of these animals failed to develop severe glomerulonephritis. Similarly, 3 of 14 mice receiving cortisone alone and 2 of 7 mice treated with cortisone and IgG also had minimal to mild glomerular lesions.

Immunopathology

Autoantibodies. All control mice and 33 of 35 treated mice, regardless of the mode of therapy, developed significant ANAB titers (1/16–1/512) during

the course of the experiment. In contrast, all control animals had anti-D-DNA by 6–7 mo of age, but only 15 of 35 mice in the treatment groups had developed circulating antibody to D-DNA before the time of sacrifice (up to 15 mo of age). Because absence of anti-denatured antibody was associated with prolonged survival time, elution studies were performed on kidneys from selected animals in the control and treatment groups.

Kidney eluates. 3 of 6 control and 7 of 14 animals from the treatment groups that developed severe glomerulonephritis during the course of the experiment contained significant levels of anti-denatured antibody in their renal eluates. Only 1 of 11 successfully treated mice with prolonged survival and minimal renal lesions was shown to have eluate antibody with specificity for D-DNA. In addition, the eluates for all control and treated mice were analyzed and found negative for antibody with specificity for the C-type viral antigen P-30.

DISCUSSION

The focal point of this paper is the demonstration that the manifestations of glomerulonephritis in murine SLE may be improved by immunotherapeutic techniques that involve the induction of tolerance to nucleic acid antigens. The best therapeutic regimen consisted of isologous IgG nucleoside conjugate (tolerogen) combined with cortisone. Two doses of cortisone spaced by a 3-wk interval were found to be necessary for the most effective action of tolerogen. Although cortisone alone, as shown by others (12), caused a temporary improvement, reduction in proteinuria and survival beyond any other control or experimental group were only achieved by the combined action of cortisone and tolerogen.

These data are in agreement with and expand preliminary observations that suggest that induction of tolerance in adult mice to polymerized flagellin is made possible by means of a cortisone-induced shift toward lower concentrations of the otherwise impracticably high antigen dose required to induce unresponsiveness (6, and see fn. 2). Recent data suggest that this antigen dose shift is under the control of cortisone-sensitive and radioresistant accessory cells (see fn. 2). Although cortisone is known to affect several populations of T- and B-lymphoid cells (19–21), the nature of the cellular mechanism by which cortisone facilitates tolerance in unknown. Cortisone, which is known to be a powerful anti-inflammatory agent, was also shown to inhibit immune complex deposition within the walls of glomerular capillaries (22). Thus, the anti-inflammatory action of cortisone and its effect on deposition of immune complexes in the glomeruli may be synergis-

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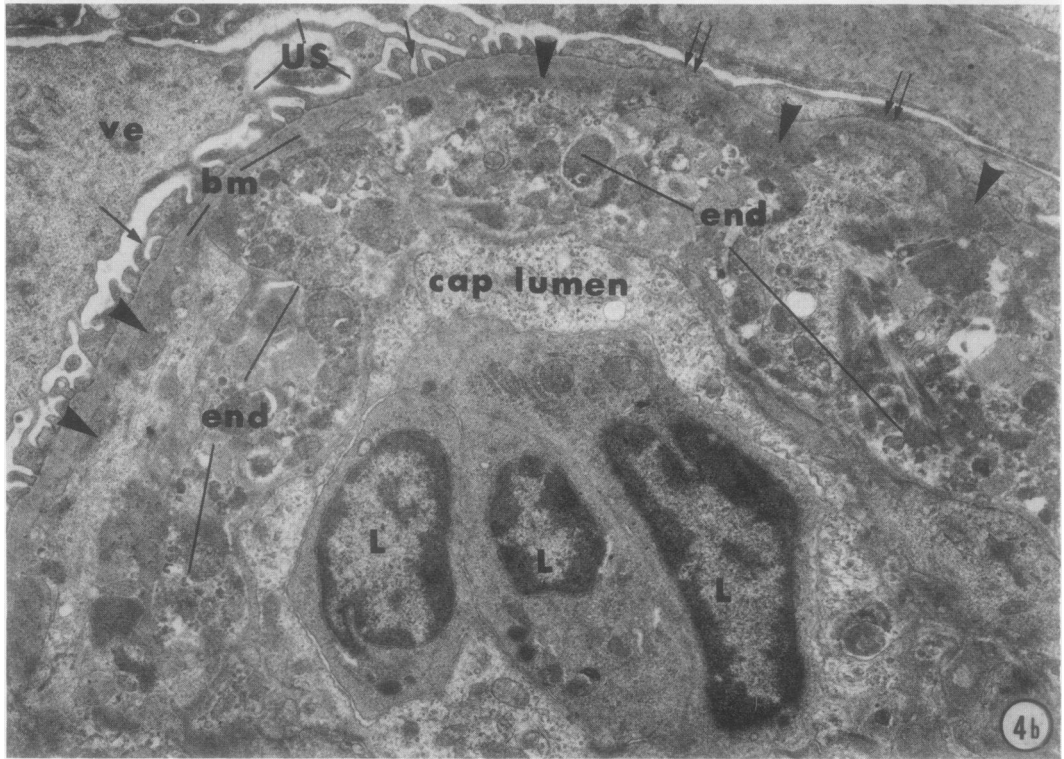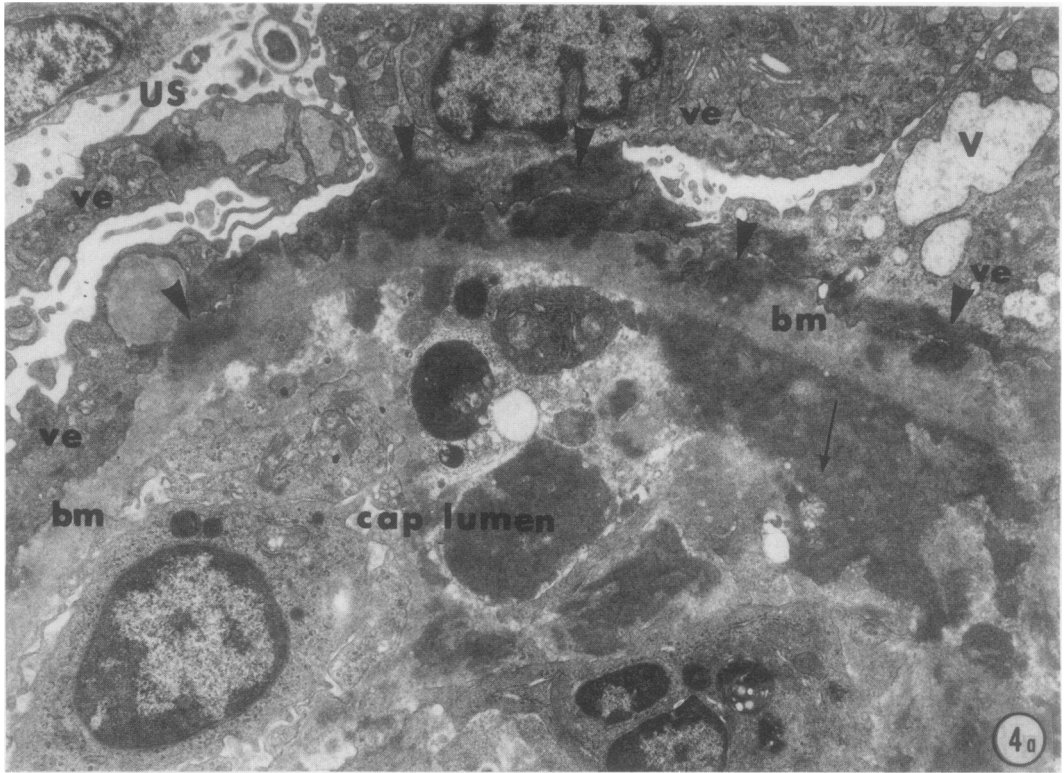
tic with tolerance induction in reducing the severity of the renal lesion in adult BWF₁ mice.

The clinical improvement exemplified by reduced proteinuria and prolonged survival was also accompanied by a reduction in circulating antibody to D-DNA in animals that were seropositive at the beginning of the treatment schedule. Animals seronegative to D-DNA at the onset of the (C + T) therapy failed to develop antibody to D-DNA during the course of treatment, indicating the efficacy of the tolerizing procedure. In contrast, cortisone treatment of seronegative animals failed to prevent the development of anti-D-DNA antibody. The presence or absence of anti-N-DNA antibody was not affected by the different treatment schedules, and almost all mice developed ANAB, suggesting that suppression of antibody to D-DNA was antigen specific. So far, our studies have involved the continuous administration of the tolerogen at biweekly intervals over a period of several months. The rationale behind this treatment rests on the assumption that self-reactive clones continuously escape because of defective homostatic control mechanisms in BWF₁ mice.

It might be argued that in these experiments the success of therapy was due to the continuous neutralization of circulating antibody to D-DNA by the repeated administration of tolerogen, rather than the reestablishment of tolerance to D-DNA. Although peripheral neutralization of circulating antibody is difficult to rule out as a basis for the successful therapy, it is unlikely because tolerogen alone did not influence survival, and the treatment with cortisone and D-DNA was not successful. Furthermore, in strains of mice other than BWF₁, tolerance to exogenous nucleic acid antigens induced by isologous IgG conjugate was shown to be long lasting (23). Recently, direct and indirect plaque-forming cells to nucleoside were also suppressed in BALB/C mice by isologous IgG nucleoside conjugate (24), demonstrating that tolerance was induced at the cellular level. A similar mechanism might occur in BWF₁ mice. In view of the

previous findings that tolerance is induced specifically to determinants on isologous IgG, and because the actual determinant for the BWF₁ antibody is probably larger than a nucleoside, it is not surprising that only partial tolerance to D-DNA was obtained.

It is interesting to note that although the number of animals is small (8), treatment with cortisone and IgG appears to have some beneficial influence on the degree of proteinuria and the rate of survival, because two of these animals failed to develop severe nephritis. Whether cortisone together with the carrier (IgG) has some nonspecific effect on the disease in unknown. However, from the serological data it is clear that this influence, if any, is not due to a feedback inhibition by isologous IgG on anti-D-DNA antibody formation.

Before a discussion of the relationship between the clinical observations and the renal lesions affected by the different treatments, it should be emphasized that the various therapeutic regimens were initiated at 6 mo of age, a time when female BWF₁ mice were already suffering significant glomerulonephritis. Thus, the legions may have reached a degree of irreversibility by this time. It is also difficult to compare the severity of the nephritis between moribund, untreated animals at 6–9 mo of age with successfully treated mice at 13–16 mo of age because many pathological events unrelated to therapy may have also taken place during the period of prolonged survival. With these limitations in mind, similarities and differences in the various treatment groups can be discussed.

Regardless of the treatment used, mice with prolonged survival times had a renal lesion distinctly different from control animals. Mild to moderate membranoproliferative glomerulonephritis was observed by light microscopy, and uniform, diffuse membranous deposits of immunoglobulin and complement were present in the immunofluorescent lesion. Ultrastructurally, the proliferative component of the lesion was outstanding, with marked hyperplasia of intraglomerular endothelial and mesangial cells and

FIGURE 4 (a) Electron microscope study. Severe glomerulonephritis: portion of a glomerular capillary loop. Mouse treated with tolerogen plus cortisone: 16 mo. The lesions are similar to those observed in untreated control animals (6–8-mo old). The capillary lumen (cap lumen) appears engorged with cells and debris. The GBM (bm) is thickened, irregular. The visceral epithelial cells (ve) are hypertrophied and contain vacuoles (V); their foot processes are fused. Numerous electron-dense deposits (thick arrows) are visible in the GBM both on its external side and on the adjacent cytoplasm of visceral epithelial cells. Submembranous electron-dense deposits (thin arrow) are also seen. ×11,500. (b) Moderate glomerulonephritis: portion of a glomerular capillary loop. Mouse treated with tolerogen plus cortisone: 16 mo. In contrast to what is seen in severe glomerulonephritis, the GBM (bm) appears thin, covered with healthy, distinct foot processes (thin arrows) of the visceral epithelial cell (ve); only a few foot processes are fused (double thin arrows) over a portion of the GBM which remains thin. The capillary lumen (cap lumen) is, however, very reduced: it is almost filled with swollen endothelial cells (end), debris, and several lymphocytes (L). Electron-dense deposits are visible (thick arrows) in a subendothelial location. Urinary space (US). ×9,200.

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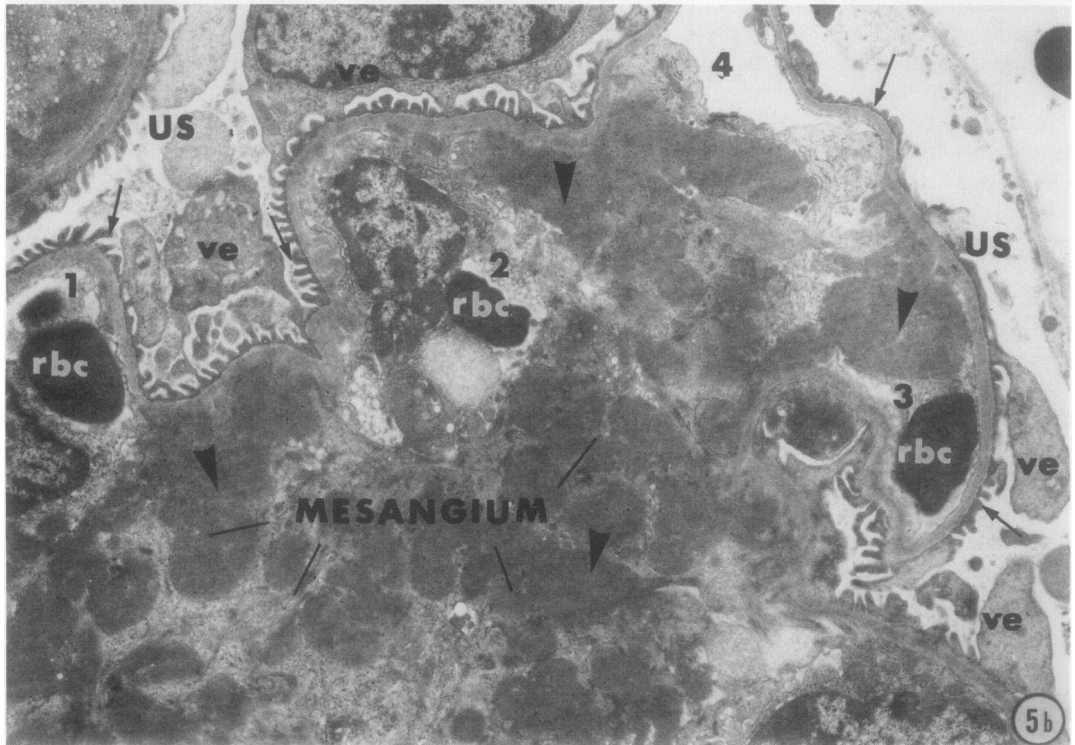
prominent periglomerular accumulation of lympho-cytes and plasma cells. Changes in the GBM were minimal and sharply restricted to mild, irregular segmental thickenings of the GBM in areas associated with focal electron densities. Thus, the absence of severe alterations in the GBM was closely associated with prolonged survival, regardless of the treatment schedule employed. Further, treatment had little effect on the proliferative component of the BWF_1 kidney lesion because moderate to marked hyperplasia of endothelial and mesangial cells was commonly encountered in this group of animals.

6 of 20 mice treated with cortisone and tolerogen failed to develop severe glomerulonephritis during the 15-mo period of observation, and in only one of these animals could antibody to D-DNA be demonstrated in eluates prepared from the renal lesion. Five other mice treated with either cortisone alone or cortisone and IgG also failed to develop severe glomerulonephritis, and their eluates were also negative for anti-DNA antibody. Thus, prolonged survival of treated mice was associated with transient or prolonged reduction in circulating antibody to D-DNA, moderate to minimal alterations of the GBM, and an absence of anti-D-DNA antibody in eluates prepared from the renal lesion.

It is important to note that some mice with prolonged survival were found to have severe glomerulonephritis at the time of necropsy. Eluates from 50% of these mice, as well as those from some control mice, also failed to react immunologically with DNA, thus indicating the possibility that antigens other than nucleic acids were responsible for the immune complex-mediated renal lesion. Viral antigens have been proposed as a possible source of antigen in SLE (25–27). Our attempts to demonstrate antibody to the P-30 viral antigen associated with murine leukemia virus in the eluates of control and test animals failed. However, a recent report (18) describes the presence of GP-70, an envelope-related antigen of murine leukemia virus, in the renal lesion of untreated BWF_1 mice. Should such an endogenous viral antigen commonly contribute to the development of glomerulonephritis in mice of this strain, then our failure to successfully treat all animals with cortisone and tolerogen could readily be explained, because the induction of tolerance to the nucleosides of DNA would only affect immunological reactivity to one of several antigens capable of inducing the glomerular lesion. In fact, analysis of the pathologic data in these experiments strongly supports the notion that multiple antigens may be responsible for the immunologically mediated nephritis of BWF_1 mice. Prolonged survival of our treated mice may well be the result of reducing the total amount of glomerular injury by eliminating one major antigenic source capable of forming soluble immune complexes. In species such as man, where defined viral antigens have yet to be identified as an integral component of the renal lesion associated with SLE, the beneficial effects of tolerance induction to nucleic acid antigens may be even more marked than we observed in the BWF_1 animal model of the disease because DNA plays a major role in the immunologically mediated destruction of tissue in human SLE (28).

In conclusion, our data offer a new approach to the treatment of preexisting glomerulonephritis in murine SLE which may involve the induction of tolerance to DNA facilitated by cortisone. One of the advantages of this therapy is its capacity to prevent development of the nephrotic syndrome and prolong survival, without the hazards of long-term steroid therapy. Another is that the specific deletion of antibodies to nucleic acid antigens may provide a useful way to examine the role of other antigens in the immuno-pathogenesis of the disease. The potential clinical implication of this observation for the treatment of SLE in humans is worth emphasizing (29). Although significant differences, not only in the specificity of the antibody to single- and double-strand DNA, but also in susceptibility to steroid effects exist between mouse and man, it appears that immunotherapy induced by autologous IgG constitutes an attractive and rational approach to the restoration of immunologic tolerance to self-DNA (30). The ultimate success of this form of therapy will depend in part on the feasibility of constructing a tolerogen capable of suppressing antibody to N-DNA in man. Although multiple antibodies to a wide variety of autologous antigens are spontaneously formed in human SLE, there is increasing evidence in both mice and man (28, 31) that IgG antibody with high avidity to N-DNA is mainly

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FIGURE 5 (a) Discrete glomerulonephritis: glomerular capillary loops. Mouse treated with tolerogen plus cortisone: 16 mo. Portions of three glomerular loops are visible, exhibiting a thin GBM covered by healthy foot processes (arrows) of the visceral epithelial cells (ve). The capillary lumens (1, 2, 3) are either free (3) or loosely filled with lymphocytes (L), red blood cells (rbc), and monocytes (M). No electron-dense deposits are seen. The urinary space (US) is visible between the capillary loops. ×6,000. (b) Discrete glomerulonephritis: mesangial zone. Mouse treated with tolerogen plus cortisone: 16 mo. Abundant electron-dense deposits (thick arrows) are visible in the thickened mesangium which compresses adjacent glomerular capillaries (1, 2, 3). Red blood cells (rbc) are seen in the lumens thus narrowed. A fourth capillary loop (4) appears open. The GBM is thin with distinct foot processes (thin arrows). US, urinary space; Ve, visceral epithelial cell. ×7,800.

responsible for tissue damage. Thus, it is conceivable that the specific suppression of a small population of antibody-producing cells with specificity to a well-defined nucleic acid antigen, combined with the known anti-inflammatory effects of cortisone, might be sufficient to curtail the development of glomerulonephritis and prevent death due to renal failure.

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