SELECTIVE SUPPRESSION OF RETROVIRAL gp70-ANTI-gp70 IMMUNE COMPLEX FORMATION BY PROSTAGLANDIN E_1 IN MURINE SYSTEMIC LUPUS ERYTHEMATOSUS*

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Mice with a spontaneous disease that approximates systemic lupus erythematosus (SLE)[1] have marked amounts of immune complexes (IC) containing the major envelope glycoprotein, gp70, of endogenous retroviruses and the corresponding antibody (1). These IC become apparent in the circulation close to the onset of disease, and concentrations rise as the disease worsens. Strains of mice without this immunologic disease do not develop gp70-anti-gp70 IC (gp70 IC), at least in detectable levels. The observation that gp70 deposits along with host immunoglobulin (Ig) and complement in the diseased glomeruli of SLE-prone mice seems to be good evidence that gp70 IC are one source of the renal lesions that contribute to these animals' deaths (2, 3). Additionally, the involvement of DNA-anti-DNA IC in the pathogenesis of murine SLE was demonstrated by the significant concentration of anti-DNA antibodies found in eluates from the diseased kidneys of these mice (3, 4). The induction of tolerance to DNA antigen in (NZB × NZW)F_1 hybrid mice (NZB × W) was followed by decreased anti-DNA production, less severe nephritis, and prolonged survival (5, 6).

We have now examined the importance of gp70 IC and anti-DNA antibody formation in the renal disease of three murine strains (NZB × W, MRL/1 and BXSB) that are SLE-prone. For this purpose, we used prostaglandin E_1 (PGE), which altered immunologically mediated renal disease and prolonged survival without affecting degree of anti-DNA formation, as established in a previous study of NZB × W mice (7, 8). Our results indicate that NZB × W and MRL/1 mice treated with PGE had far lower levels of circulating gp70 IC, amelioration of renal disease, and increased survival time compared with untreated controls. However, BXSB mice were not responsive to PGE treatment, and life span and serum levels of gp70 IC in the treated mice were essentially identical to those in untreated mice.

[1] *Abbreviations used in this paper:* AMG, aggregated murine IgG; dsDNA, double-stranded DNA; gp70 IC, retroviral gp70-anti-gp70 immune complex; IC, immune complex(es); 2-ME, 2-mercaptoethanol; MuLV, murine leukemia virus; NZB × W, (NZB × NZW)F_1 hybrid; PBS, phosphate-buffered saline; PGE, prostaglandin E_1; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA; Staph A, *Staphylococcus aureus* protein A.

Materials and Methods

Mice.   NZB × W female mice were obtained by mating NZB females with NZW males in our animal colony. MRL/1 (*lpr/lpr*) and BXSB mice originally came from The Jackson Laboratory, Bar Harbor, Maine, and were bred at Scripps Clinic and Research Foundation, La Jolla, Calif. Their blood samples were collected by orbital sinus puncture, and the sera were stored at −20°C until use.

Treatment with PGE.   PGE, which was generously supplied by Dr. J. Pike of Upjohn Company, Kalamazoo, Mich., and by Ono Pharmaceutical Company, Ltd., Osaka, Japan, was stored in absolute ethanol at −20°C and diluted daily with phosphate-buffered saline (PBS). The final solution consisting of PGE in 10% ethanol-PBS at pH 6.8 was spectroscopically analyzed to determine purity (9). Groups of 2-mo-old female mice were injected subcutaneously with 200 μg of PGE twice daily 5 d of the week and once each Saturday and Sunday throughout the course of experiment. Controls consisted of littermates receiving the vehicle alone. Groups of mice were killed bimonthly beginning at 4 mo of age or when moribund and then autospied.

Immunologic Reagents.   Retroviral gp70 of Rauscher murine leukemia virus (MuLV) were prepared as described by Strand and August (10) and goat anti-Rauscher MuLV gp70 antisera were kindly provided by Dr. J. Elder (Scripps Clinic and Research Foundation). Goat anti-feline leukemia virus antisera were obtained from the National Cancer Institute, Bethesda, Md. Rabbit anti-murine IgG antibodies were raised in rabbits by giving repeated injections of murine IgG (Miles Laboratories Inc., Elkhart, Ind.). IgG fractions of these antisera were prepared by DEAE-cellulose column chromatography. Fluorescein isothiocyanate-conjugated rabbit anti-murine IgG were obtained from Miles Laboratories, Inc., and goat anti-murine C3 antisera were purchased from N. L. Cappel Laboratories, Inc., Cochranville, Pa. Highly polymerized calf thymus DNA (type V) was purchased from Sigma Chemical Co., St. Louis, Mo. Protein A-containing *Staphylococcus aureus* bacteria treated with formaldehyde were kindly provided by Dr. M. J. Buchmeier, Scripps Clinic and Research Foundation. Protein A-free *S. aureus* bacteria were obtained from Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, Calif.

Radiolabeling Procedures.   gp70 from Rauscher MuLV and the IgG fraction of rabbit anti-murine IgG were labeled with radioactive iodine (^{125}I) by the chloramine T method (11). Single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) were labeled with ^{125}I by the method of Commerford (12). Then, ^{125}I-dsDNA was treated with S1 nuclease (Miles Laboratories, Inc.) to remove single-strand regions within dsDNA (13).

Morphologic Studies

Tissues from PGE-treated mice and controls were assessed for morphologic and immunopathologic changes by light, fluorescence, and electron microscopy as previously described (8).

LIGHT MICROSCOPY.   Sections of kidneys were fixed in Bouin's fluid and stained with hematoxylin and eosin and periodic acid-Schiff on coded slides. Renal histopathologic alterations were graded on a semi-quantitative scale by criteria adopted from Pirani and Salinas-Madrigal (14). The scale ranged from 0 to 4: 0, normal; 0.5, minimal or questionable; 1, mild; 2, moderate; 3, moderately severe; 4, severe alterations.

IMMUNOFLUORESCENCE MICROSCOPY.   Renal tissues were studied for the deposition of IgG and gp70 by direct immunofluorescence after staining with fluorescein isothiocyanate-conjugated rabbit anti-murine IgG or goat anti-Rauscher MuLV gp70. The intensity and distribution of fluorescence were graded from 0 to 4.

ELECTRON MICROSCOPY.   Kidney tissues were fixed in glutaraldehyde-paraformaldehyde, post-fixed in osmic acid, dehydrated, embedded in Epon, sectioned, and stained with uranyl acetate and lead citrate. Renal pathologic alterations in at least two glomeruli/mouse were graded on a 0–4 scale (15).

Elution of Kidney-fixed gp70 and Ig.   Renal eluates were prepared as described previously (16). Briefly, kidneys from PGE-treated and control NZB × W mice were pooled, cut into small pieces, and washed extensively with PBS overnight at 4°C. The tissues were homogenized and washed five times by centrifugation in PBS. The precipitates were resuspended in PBS that contained Mg^{++}, digested with DNase, and eluted with 2.5 M potassium thiocyanate in PBS. The eluates obtained were concentrated with a Diaflo ultrafiltration membrane (PM10; Amicon Corp., Scientific Systems Div., Lexington, Mass.) in a microfiltration system. The

amounts of anti-DNA antibodies in the eluates were determined by quantitative absorption with cyanogen bromide-activated Sepharose 4B coated with both ssDNA and dsDNA as described previously (16).

Detection of Anti-DNA Antibodies. Serum levels of anti-DNA antibodies were determined by using a modification of the Farr DNA-binding radioimmunoassay (17). To inactivate IgM anti-DNA antibodies, sera were incubated with ^{125}I-DNA and 2-mercaptoethanol (2-ME; final concentration of 0.1 M) (18). The results are expressed as a percentage of 20 ng of ^{125}I-DNA precipitated specifically after correction for nonspecific precipitation in pooled sera from immunologically normal mice of five different strains (AKR, BALB/c, C3H, C57BL/6, and DBA/2). The Ig class of anti-DNA antibodies was also analyzed by sucrose density-gradient ultracentrifugation (18).

The avidity of anti-DNA antibodies was determined by measuring the dissociation rate of preformed ^{125}I-DNA-anti-DNA complexes in the presence of unlabeled DNA (19). Tubes that contained the pooled sera, which bind ~40% of the added ^{125}I-DNA (20 ng), were incubated with ^{125}I-DNA at 4°C for 24 h. Then, a 100-fold excess of unlabeled DNA (2 μg) was added to each tube, and at increasing intervals thereafter the serum DNA-binding activity was determined as described above. Dissociation curves were then constructed by plotting log (Px/Po) × 100 against time, where Px is the fraction of ^{125}I-DNA bound at time x and Po the fraction bound at time 0. Because of the antibody heterogeneity, dissociation curves with anti-DNA antibodies were resolved into high- and low-avidity components by curve peeling.

Radioimmunoassays for Circulating DNA-Anti-DNA IC. Three different procedures were used to detect DNA-anti-DNA IC in sera. First, serum DNA-binding activity was measured before and after treatment of sera with DNase, which liberates anti-DNA antibodies bound to DNA resulting in the increase of DNA-binding activity (20). The detail of this procedure has been described previously (18). Second, the presence of rapidly sedimenting anti-DNA complexes was analyzed by the sucrose density-gradient ultracentrifugation according to the method of Bruneau and Benveniste (21). Last, [^{3}H]actinomycin D, which specifically binds DNA, was used to detect DNA bound to anti-DNA antibodies as described previously (22).

Radioimmunoassay for Circulating IC. IC in sera of all test mice were detected and quantitated with a modified Raji cell radioimmunoassay (3). Aggregated murine IgG (AMG) was used for the standard curve and the ^{125}I-labeled IgG fraction of rabbit anti-murine IgG was used to quantitate Raji cell-bound IgG. Pooled BALB/c serum stored at −70°C was the source of complement in the standard curve. Results are expressed as microgram equivalents of AMG/ml murine serum. The upper normal limit of IC in the sera of 20 μg equivalent AMG/ml was based on the mean value plus 3 SD deviations for a control group of 20 BALB/c female mice.

Radioimmunoassay for gp70 IC. To determine the amounts of gp70 bound to Ig, sera were depleted of Ig by absorption with protein A-containing *S. aureus* (Staph A). 0.3 ml of serum diluted 1/120 in borate buffer that contained 0.01% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) was incubated with 0.025 ml washed, packed Staph A for 30 min at room temperature. After the precipitation of Ig-bound Staph A by centrifugation, the amount of gp70 in the supernate was determined by inhibiting the binding of goat anti-feline leukemia virus antibody to ^{125}I-labeled gp70 from Rauscher MuLV as described previously (1). As a control, sera were treated with protein A-free Staph A. After comparing the concentrations of gp70 in both samples, the amounts of gp70 bound to Ig were calculated. The mean concentrations of Ig-bound gp70 in 20 serum samples from 2-mo-old NZB × W and MRL/1 female mice were 2.2 ± 2.5 μg/ml and 1.5 ± 2.4 μg/ml, respectively.

Sucrose Density-Gradient Ultracentrifugation. For determining the sedimentation characteristics of serum gp70, 0.2-ml samples diluted 1/4 in 0.01 M PBS, pH 7.0, were layered on 5–20% (wt: vol) linear sucrose density gradients in PBS. Samples were then centrifuged at 36,000 rpm for 15 h at 4°C with a SW60 rotor in a Beckman L-75 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). The positions of IgM, IgG, or gp70 were established by radioactive markers, then the gradients were divided into 12 fractions. Each fraction was radioimmunoassayed for the presence of gp70.

Quantitation of Serum Levels of C3 and Ig. Serum levels of C3, IgG1, IgG2a, and IgG2b were measured by radial immunodiffusion in agar according to the method of Mancini et al. (23). Serum C3 levels are expressed as a percentage of the normal pool values.

Statistical Analysis.   Statistical analysis was performed with the Mann-Whitney U-test and the Wilcoxon two-sample test.

Results

Effect of PGE on Survival Rate and Renal Disease in NZB × W Female Mice.   NZB × W female mice were treated with PGE beginning at 2 mo of age. As reported (8), 50% of the control females, which did not receive PGE, died by 10.2 mo of age and only 10% were alive at 1 yr. However, none of the mice treated with PGE died within the year.

When evaluated for renal histopathology after killing, PGE-treated mice had less evidence of IC glomerulonephritis than controls throughout the experiments. Representative values from 10-mo-old mice are shown in Table I. As determined by light microscopy, control animals had typical glomerular lesions characteristic of this strain. These included an expansion of the mesangium caused by an increase in the number of cells and a thickening of the peripheral capillary loops. In contrast, animals treated with PGE had only minimal alterations. By immunofluorescence, larger amounts of Ig and retroviral gp70 accumulated in glomeruli of control mice as compared with the PGE-treated group. This finding was further substantiated by electron microscopy (Table I): control mice had prominent electron-dense deposits in both the mesangial areas and the peripheral capillary loops. In comparison, PGE-treated mice had significantly fewer deposits, which were confined to the mesangium.

A considerable amount of gp70 (19.0 μg/g of tissue) was demonstrable in renal elutes from 1-yr-old control NZB × W female mice, whereas renal elutes from PGE-treated mice of the same age and sex contained significantly less gp70 (6.2 μg/g of tissue), identical to the amount presumably present in the tubular brush borders of 2-mo-old healthy mice (5.0 μg/g of tissue). In keeping with the reduced IC deposition in the glomeruli, the amount of IgG elutable from kidneys of PGE-treated mice was 72 μg/g of tissue, only ~10% of that found in untreated controls (667 μg/g of tissue). In spite of the reduced IgG deposition, PGE-treated mice had 11 μg anti-DNA antibodies/g kidney vs. control levels (30 μg/g kidney). When considered in terms of specific activity, at the same concentration of IgG, the ssDNA- and dsDNA-binding activities of renal eluates from PGE-treated mice were two and three times greater, respectively, than those of untreated mice. Notably, anti-DNA antibodies were not detected in kidney homogenates of 2-mo-old mice.

Anti-DNA Antibodies and Circulating IC in PGE-treated and Control NZB × W Female Mice.   There were essentially no differences in the serum levels of anti-ssDNA and anti-dsDNA antibodies, and IgG in 4-mo-old and 10-mo-old PGE-treated mice and

TABLE I

Microscopic Studies of Kidneys in PGE-treated and Control 10-mo-old NZB × W Female Mice

	PGE	Saline
Light microscopy	1.1 ± 0.2*	3.5 ± 0.2
Fluorescence microscopy		
IgG	1.2 ± 0.5	3.1 ± 0.2
gp70	0	1.4 ± 0.2
Electron microscopy	1.2 ± 0.2	3.2 ± 0.3

* Grades of renal pathology. Mean ± SEM (10 mice in each group).

controls, despite the great difference in their survival rates and degree of renal destruction. Representative results of serum ssDNA- and dsDNA-binding activity in 8-mo-old animals of both groups are shown in Fig. 1. To establish the class of anti-ssDNA and anti-dsDNA antibodies, we added 2-ME, which inactivates IgM, to the mixtures being tested for ssDNA and dsDNA binding activities. However, PGE-treated and control mice were equally resistant to 2-ME treatment throughout the test period (Table II). Additionally, pooled sera from 4- and 6-mo-old PGE-treated mice subjected to sucrose density-gradient ultracentrifugation had essentially identical levels of IgM and IgG anti-ssDNA, as well as anti-dsDNA antibodies as sera from untreated mice of the same age and sex (data not shown). This analysis of the anti-DNA Ig class indicates the similar age-dependent development of IgG antibodies to ssDNA and dsDNA in the PGE-treated and untreated animals.

The avidity of anti-ssDNA antibodies in pooled sera of 8-mo-old NZB × W female mice in PGE-treated and control groups was then compared by determining the relative dissociation rates. However, both groups of sera were essentially identical in this respect (Table III). Thus, the treatment of NZB × W mice with PGE did not
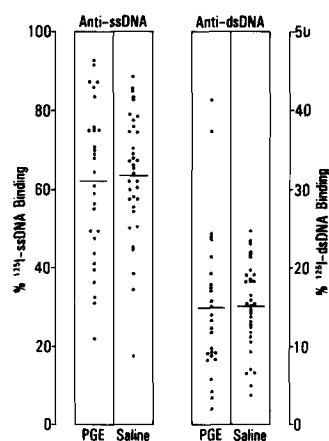

Fig. 1.   Serum ^{125}I-DNA binding activity in 8-mo-old PGE-treated and control NZB × W female mice. Bars represent the mean value of each examination.

Table II

Sensitivity of Anti-ssDNA and Anti-dsDNA Antibodies to 2-ME in PGE-treated and Control NZB × W Female Mice

Group	Age	^{125}I-ssDNA binding*		^{125}I-dsDNA binding*	
		Without 2-ME	With 2-ME	Without 2-ME	With 2-ME
	mo	%		%	
PGE	5	33.7 ± 10.4‡	34.6 ± 16.3	12.7 ± 4.9	12.8 ± 5.4
Saline	5	32.2 ± 14.9	29.3 ± 15.6	10.3 ± 4.4	10.0 ± 5.1
PGE	8	55.7 ± 13.5	59.7 ± 17.5	21.5 ± 7.3	25.8 ± 10.3
Saline	8	53.9 ± 10.6	57.4 ± 19.0	18.3 ± 5.3	22.8 ± 8.1

* 10 μl of serum was incubated with 20 ng of ^{125}I-DNA with or without 0.1 M 2-ME.
‡ Mean of 10 mice ± 1 SD.

TABLE III

Avidity of Anti-ssDNA in PGE-treated and Control NZB × W Female Mice

Serum*	Avidity‡ index	Avidity component§				Avidity component‖			
		High		Low		High		Low	
		Percent of $t_{1/2}$	min	Percent of $t_{1/2}$	min	Percent of $t_{1/2}$	h	Percent of $t_{1/2}$	min
PGE	4.19	84	>100	16	1.5	61	>48	39	24
Saline	4.37	84	>100	16	1.2	63	>48	37	24

* Pooled sera from 10 8-mo-old mice.

‡ Avidity index = $\left(\dfrac{1}{\% \text{ dissociation at 30 min.}} \right) \times 100.$

§ Resolution of the dissociation curves into the high- and low-avidity components from data points obtained over 30-min dissociation periods.

‖ Resolution of the dissociation curves into the high- and low-avidity components from data points obtained over 30-h dissociation periods.

appear to alter either the quality or the quantity of anti-DNA antibodies, although PGE was effective in delaying glomerulonephritis and prolonging this strain's survival.

The presence of DNA-anti-DNA IC in sera of PGE-treated and control NZB × W mice was investigated by three different methods that can efficiently detect DNA-anti-DNA IC in sera (20–22). However, none of the techniques demonstrated measurable amounts of DNA-anti-DNA IC in sera from either groups of mice throughout the course of experiments (data not shown).

However, when serum levels of IC detectable by Raji cell assay were compared in PGE-treated and control mice, tested at 8-mo of age, concentrations were abnormally high in both groups. The control NZB × W mice had mean IC levels of 126 μg with 100% of the animals having values above normal. Comparatively, the PGE-treated mice had a somewhat lower but still increased IC concentrations of 88 μg, representing 93% of animals above normal.

Although the PGE-treated mice had considerable amounts of circulating IC, serum concentrations of C3 were still high. In the 8-mo-old mice, mean values were 81.0 ± 17.4% (± 1 SD) of normal pooled values, which were comparable to those of 2-mo-old NZB × W mice (96.3 ± 19.1%), indicating no significant consumption of complement in the PGE-treated mice. By contrast, serum levels of C3 were greatly suppressed in the untreated mice. The mean values of 8-mo-old control NZB × W mice were only 43.2 ± 14.1%.

Retroviral gp70 IC in PGE-treated and Control NZB × W mice. We previously demonstrated (1) that only sera from mice of SLE-prone strains, including NZB × W mice, contain a rapidly sedimenting heavy form of gp70 complexed to Ig, that the appearance of these IC parallels the onset of renal disease and that the complexes persist throughout the course of disease. Therefore, we next examined the presence of circulating gp70 IC in the PGE-treated mice by determining the sedimentation rate of serum gp70 and by quantitating absorption of Ig-complexed gp70 with Staph A.

After sucrose density-gradient ultracentrifugation, pooled sera from 5-mo-old control NZB × W mice contained significant amounts of gp70 in fractions that contained material heavier than 7S IgG (Fig. 2). In pooled sera from 8-mo-old control mice, even larger amounts of gp70 appeared in the heavy fractions. In contrast, of NZB ×
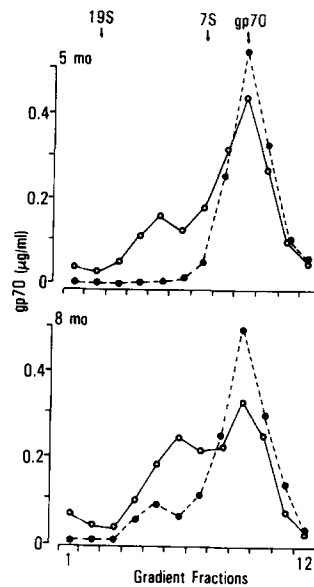
Fig. 2.   Sedimentation rate of serum gp70 from PGE-treated (●) and control (O) NZB × W female mice at 5 and 8 mo of age analyzed by sucrose density-gradient ultracentrifugation. The concentration of gp70 was determined by the radioimmunoassay. The positions of markers are indicated by arrows.
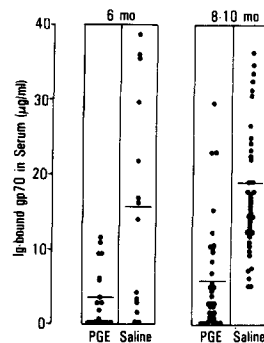

Fig. 3.   Serum concentrations of Ig-bound gp70 in PGE-treated and control NZB × W female mice as determined by radioimmunoassay combined with Staph A absorption. The mean values are indicated by the horizontal line.

W mice treated with PGE, the heavy form of gp70 was detectable only in the 8-mo-old group. Even then, less heavy gp70 was apparent than in untreated NZB × W mice of the same age.

To compare the levels of gp70 IC in PGE-treated and control mice more quantitatively, we measured amounts of gp70 present before and after Ig was removed from the sera with Staph A. The quantities of Ig-bound gp70 were significantly higher in untreated control mice than in those treated with PGE (Fig. 3 and Table IV). As a result of absorption, in the control group 64% of sera from 6-mo-old mice and >90% of the sera from 8- to 10-mo-old mice lost more than one-fifth of their gp70 content. In contrast, when tested at 6, 8, and even 10 mo of age, <20% of the PGE-treated mice had significant amounts (more than one-fifth) of gp70 complexed with Ig. It

TABLE IV

Serum Levels of Total gp70 and Ig-bound gp70 in PGE-treated and Control NZB × W Female Mice

Treatment	Age	n	Total gp70*	Ig-bound gp70*	Percent of Ig-bound gp70 of total gp70
	mo		µg/ml	µg/ml	
PGE	6	18	39.3 ± 11.1	3.6 ± 4.2‡	9.3 ± 11.3§
Saline	6	14	37.7 ± 12.6	15.7 ± 14.4‡	36.7 ± 27.4§
PGE	8–10	34	50.2 ± 15.3	5.9 ± 7.4‖	11.8 ± 12.8‖
Saline	8–10	43	45.7 ± 16.9	19.1 ± 10.8‖	41.1 ± 14.2‖

* All the values represent mean ± 1 SD.
‡ $P < 0.01$.
§ $P < 0.005$.
‖ $P < 0.001$.

should be noted that the total concentrations of gp70 detectable in sera were similar in treated and control mice (Table IV).

To examine the possibility that PGE present in sera from the treated mice might have interfered with the absorption of Ig-bound gp70 by Staph A, serum samples from 8-mo-old NZB × W mice containing considerable amounts of Ig-complexed gp70 were absorbed with Staph A in the presence of various concentrations of PGE (0.001–100 µg/ml in serum). Regardless of the concentrations tested, PGE did not interfere with the absorption of gp70 IC by Staph A (data not shown).

Effect on PGE on MRL/1 and BXSB Mice.   The effect of PGE on the development of the renal disease, anti-DNA antibodies and gp70 IC was then tested in two newly developed strains, MRL/1 and male BXSB mice, which are predisposed to SLÉ. Both strains received PGE injections beginning at 2 mo of age, just as the NZB × W mice did. The effect of PGE on MRL/1 mice was dramatic; survival times was markedly prolonged (at 8 mo of age, 90% of the PGE-treated female mice were alive—all of the female controls were dead), and the development of glomerulonephritis was greatly suppressed. The retardation of renal disease was reflected in histologic grades; the mean grades of controls were 3.7, whereas treated mice reached a mean of only 1.1. In addition, the treatment prevented lymphoproliferation compared to that in controls (Table V). However, there was no significant difference in serum levels of anti-DNA antibodies, although slightly less IgG1 was observed in the PGE-treated mice (Table V). The details of this experiment will be described elsewhere. (V. E. Kelley, A. Winkelstein, S. Izui, and F. J. Dixon. Manuscript in preparation.) When the two groups of sera were compared for the presence of Ig-complexed gp70, the incidence and quantities of gp70 IC were significantly higher in untreated mice than in the PGE-treated mice (Table VI). 75% of sera from 4-mo-old untreated female mice had significant amounts (more than one-fifth) of gp70 complexed with Ig, but only 25% of sera from the PGE-treated mice contained significant amounts of Ig-bound gp70. Results were similar in male MRL/1 mice (Table VI). Substantial amounts (more than one-fifth) of gp70 bound to Ig were observed in 55% of sera from 4-mo-old untreated male mice, compared with only 12% of the PGE-treated mice. Nevertheless, such treatment did not significantly affect the total serum levels of gp70.

TABLE V

Effect of PGE Treatment on Lymphoadenopathy and Serum Levels of IgG and Anti-DNA in MRL/l Female Mice

	PGE	Control
Lymphoadenopathy	2/20	20/20
IgG‡		
IgG1	2.99 ± 2.18§	6.42 ± 1.56§
IgG2a	11.69 ± 2.96‖	13.76 ± 5.00‖
IgG2b	1.05 ± 0.35‖	1.59 ± 0.88‖
Anti-dsDNA§	12.2 ± 7.5‖	14.5 ± 9.8‖
Anti-ssDNA‡	41.9 ± 20.4‖	49.1 ± 17.4‖

* Number positive/number tested up to 4 mo of age.
‡ Serum levels of IgG (mg/ml) and anti-DNA antibodies were determined in 4-mo old animals. Anti-dsDNA activity is expressed as the mean percentage of 20 ng ^{125}I-DNA precipitated specifically by 10 µl serum. Anti-ssDNA activity is expressed as the mean percentage of 20 ng ^{125}I-DNA precipitated by 2 µl serum. Results indicate mean values ± 1 SD of 20 PGE-treated and 17 control mice.
§ $P < 0.01$.
‖ $P > 0.1$.

TABLE VI

Serum Levels of Total gp70 and Ig-bound gp70 in PGE-treated and Control 4-mo-old MRL/l Mice

Treatment	Sex	n	Total gp70*	Ig-bound gp70*	Percent Ig-bound gp70 of total gp70
			µg/ml	µg/ml	
PGE	Female	20	17.7 ± 9.3	3.4 ± 5.0‡	18.6 ± 18.8‡
Saline	Female	17	21.0 ± 9.5	10.0 ± 9.9‡	38.6 ± 25.7‡
PGE	Male	17	22.8 ± 10.5	1.4 ± 2.6§	6.5 ± 10.5‡
Saline	Male	20	26.9 ± 12.3	9.2 ± 8.1§	30.4 ± 26.2‡

* All the values represent mean ± 1 SD.
‡ $P < 0.005$.
§ $P < 0.001$.

In contrast, the BXSB males remained unchanged by treatment with PGE in respect to survival or development of renal disease. One-half of the PGE-injected and untreated BXSB mice alike died of glomerulonephritis before 6 mo of age. Their serum levels of gp70 IC at 4–5 mo of age were essentially identical: PGE-treated 5.7 ± 3.9 µg/ml, untreated 6.3 ± 5.7 µg/ml, $P > 0.1$.

Discussion

In NZB × W mice, PGE modulates the expression of SLE (7, 8). The development of IC glomerulonephritis is suppressed, and survival is greatly prolonged. PGE also prevents the development of fatal glomerulonephritis and massive lymphoprolifera- tion that accompanies the SLE of MRL/1 mice, but does not protect BXSB mice from similar lupus-like disease.

Despite the beneficial effects of PGE in lessening renal pathology and lengthening life span in NZB × W and MRL/1 mice, their production of anti-ssDNA and anti-

dsDNA remains virtually unchanged. Because the qualitative and quantitative aspects of the anti-DNA response in murine SLE may be equally important in provoking renal disease, PGE might function by suppressing the formation of a specific subpopulation(s) of anti-DNA antibodies that is more pathogenic than others. In fact, recent studies suggest that SLE strains of mice develop new clonotypes of anti-DNA antibodies coinciding with the onset of renal disease (24). Further, the avidity and isotype of anti-DNA antibodies may be as important as their quantities in the development of tissue lesions in patients with SLE as well as lupus mice (25–27). However, we found no qualitative differences in the isotypes or avidities of serum anti-DNA antibodies in PGE-treated and untreated mice. Apparently, PGE achieves its therapeutic effect through mechanisms that do not directly involve the formation of anti-DNA antibodies. It should be noted that other forms of therapy that can prolong survival in NZB × W mice also act without altering anti-DNA levels (28–30).

The most significant observation in the present study is that the incidence and quantity of Ig-complexed gp70 are lower in the sera of NZB × W and MRL/1 mice treated with PGE than in those of untreated mice. This was demonstrated by measuring amounts of the heavy form of serum gp70, which is composed of gp70 complexed with anti-gp70 antibodies (1), and by the quantitative absorption of serum gp70 IC with Staph A, which effectively removes both IC and IgG from serum (31, 32). Such decreases in serum gp70 IC were found in PGE-treated mice throughout the course of these experiments. Adding PGE directly to sera that contained gp70 IC did not affect our ability to quantitate the free or complexed forms of gp70, thereby ruling out the possible interference of PGE in this assay. Of course, PGE-treated mice might develop as many gp70 IC as untreated mice but may clear these IC selectively from circulating blood by activating the mononuclear phagocytic systems. However, it is difficult to conceive that gp70 IC alone are cleared from the circulation, because relatively large amounts of other IC are present in sera from the PGE-treated mice.

This study clearly shows that the decreased quantities and incidences of circulating gp70 IC in the PGE-treated mice correlate well with the reduced deposition of IC in the glomeruli. In the kidneys of PGE-treated SLE mice, as in immunologically normal mice (33), gp70 was seen by direct immunofluorescence only in the tubules, but in untreated SLE mice, glomeruli as well as tubules were involved (2, 3). The amount of gp70 eluted from kidneys of 1-yr-old PGE-treated mice was one-third that from the control mice but similar to that from young healthy mice of the same strain and sex. The low complement consumption in PGE-treated mice, documented by the high C3 levels in their sera, may have resulted from the reduced levels of circulating and kidney-bound gp70 IC. This association of circulating gp70 IC with renal disease strongly suggests the importance of gp70 IC in the development of fatal glomerulonephritis in murine SLE. Furthermore, serum levels of gp70 IC were not decreased in PGE resistant BXSB mice, a fact that supports the primary involvement of gp70 IC in the renal lesions of SLE mice.

Our observation, however, does not preclude the involvement of IC other than gp70 IC in the renal disease of SLE strains. These mice produce large amounts of unidentified IC-like materials that are detectable by the Raji cell assay, the conglutinin-binding assay, and the anti-C3 solid-phase radioimmunoassay (3, 34, 35). Although the PGE-influenced decrease in serum levels of IC may result at least partly from actual depression of gp70 IC levels, PGE may also suppress the formation of

other forms of pathogenic IC such as DNA-anti-DNA IC by preventing the release of nuclear DNA antigens in circulating blood or in extravascular spaces without affecting the production of anti-DNA antibodies. The renal eluates from PGE-treated mice contained less, but still considerable amounts of anti-DNA antibodies. Moreover, anti-DNA antibodies in these eluates were more concentrated than those from untreated mice, apparently resulting from reduced deposition of other types of IC such as gp70 IC. The comparatively lesser amounts of DNA-anti-DNA IC in PGE-treated mice might also be a result of a reduction in renal damage caused by other IC, resulting in the decreased release of DNA locally in glomeruli. Finally, one cannot exclude the possibility that PGE may alter renal hemodynamics and thereby reduce the deposition rate of circulating IC (36).

The mechanisms by which PGE diminishes gp70 IC formation in circulating blood have not been defined. However, the possibility that PGE lessens the expression of xenotropic viral gp70 in serum is unlikely because serum levels of total gp70 in PGE-treated mice are as high as in untreated mice. Of course, one cannot exclude that PGE might inhibit the production of a unique and as yet undiscovered form of retroviral gp70 that may be responsible for the formation of gp70 IC. However, recent studies (S. Izui, J. H. Elder, P. J. McConahey, and F. J. Dixon. Manuscript in preparation.) of tryptic peptide maps of gp70 isolated from the circulating IC revealed its xenotropic nature, i.e., as not being different from the xenotropic gp70 normally found in sera of all SLE-prone and normal mice (37). This indicates that the formation of gp70 IC in SLE mice does not involve unique gp70, but instead results from the unique ability to make antibody to this ubiquitous molecule. Therefore, it is more likely that PGE inhibits the immune response to this endogenous viral antigen. The probability that PGE modulates immune responsiveness to xenotropic viral gp70 is exemplified by the relative absence of lymphoid hyperplasia in PGE-treated NZB × W and MRL/1 mice (38, 39). Moreover, PGE exerts its immunosuppressive effect in a somewhat selective manner, because only IgG1 is suppressed in PGE-treated MRL/1 mice without affecting IgG2a production. It should be noted that such selective suppression is compatible with the observation that serum levels of anti-DNA antibodies are unchanged by PGE treatment, because most of anti-DNA antibodies in MRL/1 mice belong to IgG2a subclass (18). Considering that PGE seems to decrease anti-gp70 antibody formation, these anti-gp70 antibodies might belong mainly to the IgG1 subclass in MRL/1 mice. If so, immune responsiveness to xenotropic viral gp70 must be regulated by a different mechanism than governs anti-DNA antibody production and, thereby, PGE could selectively prevent the formation of anti-xeno-tropic viral gp70 antibodies. Indeed, the separation of anti-DNA formation and gp70 IC formation in mice with SLE is supported by the following evidence. First, BXSB mice, which have only slightly elevated levels of anti-DNA antibodies, develop gp70 IC earlier than NZB × W mice whose much higher levels of anti-DNA antibodies appear early in life (3). Second, in preliminary studies, MRL/1 mice infected with lactic dehydrogenase virus have ~10 times fewer anti-DNA antibodies than uninfected control mice, but both have similar levels of gp70 IC. Consequently, independent regulatory mechanisms may be responsible for these two immune responses.

Summary

The effect of pharmacologic quantities of prostaglandin E_1 (PGE) was investigated in three strains of mice (NZB × NZW, MRL/1, and BXSB) that spontaneously

develop lupus-like glomerulonephritis. PGE-treatment prolonged survival and re-tarded the glomerular deposition of immune complex (IC) and the development of glomerulonephritis in NZB × NZW and MRL/1 mice, but did not similarly protect BXSB mice. Changes in the responsive strains correlated well with reduced amounts of circulating gp70 complexed with anti-gp70 antibodies compared with untreated controls, although total concentrations of gp70 (free and complexed) detectable in sera were similar in both groups of mice. The results strongly suggest that: (a) PGE selectively suppressed the immune response to retroviral gp70, (b) PGE had little effect on the quantity or quality of anti-DNA antibodies but did reduce the deposition of anti-DNA containing IC in the kidneys, and (c) gp70 IC appear to play an important role in the pathogenesis of glomerulonephritis in murine systemic lupus erythematosus.

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