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Attenuation of immune complex nephritis in NZB/W F_1 mice by a prostacyclin analogue

Y. UTSUNOMIYA, M. OGURA, T. KAWAMURA, T. MITARAI*, N. MARUYAMA† & O. SAKAI
*Department of Internal Medicine, Jikei University School of Medicine, Tokyo, *Department of Internal Medicine, Saitama Medical Centre, Saitama Medical School, Saitama, and †Department of Molecular Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*

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SUMMARY

Although prostaglandins have been shown to inhibit the evolution of the nephritis in NZB/W mice, the mechanisms of this effect are unknown. To characterize such inhibition, we injected the prostacyclin (PGI_2) analogue, beraprost, into NZB/W mice, using 0·5 mg, 1·0 mg or 5·0 mg beraprost/kg body weight of test animals three times in 1 week when the mice were 2 months old. Evaluation included measurement of urine albumin excretion, serological parameters and splenic T cell subset, as well as examination of renal histology by light and fluorescence microscopy. Mice given beraprost showed a marked decrease in urine albumin excretion and in glomerular hypercellularity compared with untreated controls. Maximal beneficial effects occurred when the dose was 5·0 mg/kg of beraprost. These effects correlated with a reduction of immune complex deposition in glomeruli. In addition, beraprost reduced serum levels of immunoglobulins and anti-double-stranded DNA antibodies, and decreased the number of helper ($L3T4^+$) T cells in splenocytes. These results indicate that beraprost attenuates the nephritis of NZB/W mice, and that the source of this effect is the reduced production of autoantibodies and deposition of immune complexes in glomeruli.

Keywords systemic lupus erythematosus nephritis NZB/W mice prostaglandin prostacyclin

INTRODUCTION

Agents used to treat renal disease are often pretested in New Zealand Black/White (NZB/W)F_1 female mice, because these animals spontaneously develop an immune complex glomerulonephritis similar to systemic lupus erythematosus (SLE) nephritis in humans [1–3]. Prostaglandin-E_1 (PGE_1) has been shown to delay the onset of proteinuria and of immune complex nephritis, as well as prolonging the survival of these mice [4–6]. Furthermore, PGE therapy lowered the amounts of immune complexes circulating in the blood and deposited in the glomeruli of humans with lupus nephritis [7]. Recently, another prostaglandin metabolite, prostacyclin (PGI_2), was also tested for the ability to ameliorate nephritis in NZB/W mice and the anti-Thy-1 glomerulonephritis in rats [6,8]. PGI_2 functioned as an anti-platelet [8,9], anti-inflammatory [10,11] and vasodilating agent [12,13], but its specific effects on humoral and cellular immunity were not identified.

In the present study, we examined the effects of a stable PGI_2 analogue (beraprost) on the proteinuria, serum autoantibody levels, splenic T cell subset and renal morphology in NZB/W mice, and defined the mechanisms by which PGI_2 modified the related renal disease.

Correspondence: Yasunori Utsunomiya MD, Department of 2nd Internal Medicine, Jikei University School of Medicine, 3-19-18 Nishi-shinbashi, Minato-ku, Tokyo, 105 Japan.

MATERIALS AND METHODS

Animals

Forty NZB/W F_1 female mice were purchased from Schloss-River Laboratories (Yokohama, Japan). All mice used in this study were maintained under standard laboratory conditions.

Reagents

The stable prostacyclin (PGI_2) analogue, beraprost (sodium-2, 3, 3a, 8b-tetrahydro-2-harks-1-[(E)-(3S)-3-hydroxy-4-methyl-1-octen-6-ynyl]-1H-cyclopenta[b] benzofuran-5-butyrate) was generously provided by the Toray Medical Company (Tokyo, Japan). Mouse albumin (Fraction V) and calf thymus double-stranded DNA (dsDNA) were obtained from Sigma Chemical Co. (St Louis, MO). Antibodies used in this study were rabbit anti-mouse albumin polyclonal antibody and goat anti-Rauscher

murine leukaemia virus glycoprotein 70 (MuLV gp70) polyclonal antibody made as described [14,15]. FITC-labelled isotype-specific goat anti-mouse immunoglobulin and C3 antibodies as well as peroxidase-labelled goat anti-mouse immunoglobulin antibody were from Cappel Laboratories (West Chester, PA). FITC-labelled donkey anti-goat IgG antibody (absorbed with mouse serum) was from Chemicon International, Inc. (Temecula, CA). FITC-labelled rat anti-mouse CD4 MoAb (GK1.5) and PE-labelled rat anti-mouse CD8 MoAb (53-6.7) were from Becton Dickinson (San Jose, CA).

Experimental protocol

At 2 months of age, NZB/W F_1 mice were injected subcutaneously three times in 1 week with either 0·5 mg beraprost/kg body weight ($n = 10$), 1 mg/kg ($n = 10$), or 5 mg/kg ($n = 10$) or the same amounts of saline ($n = 10$; controls). At 2, 6 and 8 months of age urine and serum samples were collected and tested, respectively, for albumin excretion or for immunoglobulin values and anti-dsDNA antibodies. Urine and sera were pooled from 10 mice per group and stored at −70°C until assay. At 8 months old the animals were killed to obtain tissues for histological examination of kidneys and analysis of T cell subset in spleens by fluorescence cytometry.

Single radial immunodiffusion method

The concentrations of albumin in urine and levels of immunoglobulin in sera were determined by single radial immunodiffusion (SRID) using antibodies specific for mouse albumin or mouse immunoglobulins as described previously [16].

Immunohistology

Kidney specimens were embedded in OCT compound (Miles Scientific, Naperville, IL) and quickly frozen in dry ice and acetone at −70°C. Cryostat sections were cut 3–4 μm thick and rinsed in PBS pH 7·2 three times for 15 min. Mouse immunoglobulins and C3 were stained by the following direct immunofluorescent method. The sections were incubated with FITC-labelled goat anti-mouse IgA, IgG, IgM and C3 antibodies diluted 1 : 40 in PBS for 60 min at 37°C, rinsed in PBS and mounted in *p*-phenylenediamine (PD) (Sigma)-PBS-glycerin [17]. Sections were stained for MuLV gp70 by an indirect immunofluorescent method in which incubation for 60 min at 37°C with goat anti-Rauscher MuLV gp70 serum (1 : 100 in PBS) was followed by rinsing in PBS and incubation for 60 min at 37°C with FITC-labelled donkey anti-goat IgG antibody (1 : 50 in PBS). The sections were again rinsed in PBS, then mounted in PD-PBS-glycerin and observed under a Zeiss Axiophot fluorescent photomicroscope. The relative amount of staining was scored semiquantitatively from 0 to 3 as follows: 0, negative stain; 1·0, slight staining; 2·0, moderate staining; 3·0, marked staining.

Light microscopy

For light microscopy, tissue was fixed in 10% buffered formaldehyde and embedded in paraffin. Kidney sections were stained with PAS, after which the number of cells were counted in each of more than 50 glomeruli in each section. The number of cells shown represents means ± s.d. of the mean from 6–10 mice per group analysed individually.

ELISA for dsDNA antibodies

Ninety-six-well flat-bottomed microtitre assay plates (Falcon Pro-Bind Assay Plate; Becton Dickinson, Lincoln Park, NJ) coated with 50 μl/well of 20 μg/ml calf thymus dsDNA in 0·05 M sodium carbonate buffer pH 9·6 were incubated overnight at 4°C. The wells were then washed four times with PBS/0·05% Tween 20 (PBS–T) and reactivity was blocked for 1 h at 37°C by adding 50 μl/well PBS pH 7·2 containing 1% skim milk powder (PBS–skim milk). After washing with PBS–T, sample sera from each treatment group at 1 : 500 dilution in PBS–skim milk and PBS as blanks were added to the wells in a volume of 50 μl in triplicate and incubated at 37°C for 1 h. The plates were washed with PBS–T and incubated for 1 h at 37°C with 50 μl/well of peroxidase-labelled goat anti-mouse immunoglobulin antibody diluted 1 : 1000 in PBS–skim milk. After another wash with PBS–T, the residual peroxidase was reacted with H_2O_2 and *o*-phenylenediamine dihydrochloride (Sigma) at room temperature for 20 min. The reaction was stopped with 50 μl/well of 6 N H_2SO_4. The absorbance of each well at 492 nm was measured by an MTP-32 microplate reader (Corona Electric Co., Ibaragi, Japan).

Fluorescence cytometry

One-step immunofluorescence staining was performed by using 10^6 spleen cells taken from five mice per group, then purified by centrifuging on M-SMF (Nippon Koutai Inst., Takasaki, Japan) and incubating these cells with FITC-labelled anti-L3T4 (GK 1.5) and PE-labelled anti-Lyt-2 (3.155) antibodies for 30 min at 4°C. Samples were analysed on a flow cytometer (FCM-1; Nippon Bunkou, Tokyo, Japan).

Statistical analysis

Results were expressed as means ± s.d. Within group comparisons were performed using Student's *t*-test, while comparisons of more than two groups used ANOVA test. $P < 0·05$ was considered statistically significant.

RESULTS

Effect of PGI_2 (beraprost) on urinary albumin excretion by NZB/W F_1 mice

Before treatment, less than 30 μg/ml of urinary albumin was excreted at 2 months of age by saline-treated controls and beraprost-treated NZB/W mice, as shown in Fig. 1. However, these controls manifested an aggressive increase in urinary albumin excretion at 6 and 8 months old (3·4 ± 0·6 and 10·3 ± 1·2 mg/ml, respectively). In contrast, the amounts of urinary albumin decreased in beraprost-treated mice at 6 months old. These amounts were less than 30 μg/ml in mice given 0·5 mg beraprost/kg body weight, 0·32 ± 0·03 mg/ml after 1·0 mg beraprost/kg, and 0·18 ± 0·02 mg/ml after 5·0 mg beraprost/kg. In addition, urinary albumin excretion in beraprost-treated mice at 8 months remained lower than in controls in dose-related increments (mean ± s.d.: 3·7 ± 1·0 in 0·5 mg beraprost/kg, $P < 0·01$ *versus* controls; 1·8 ± 0·2 in 1·0 mg beraprost/kg, $P < 0·001$ *versus* controls; 0·05 ± 0·01 in 5·0 mg beraprost/kg, $P < 0·001$ *versus* controls) (Fig. 1).

Renal histopathology

The histologic appearance of kidneys in the beraprost-treated mice and controls was assessed when they reached 8 months of age. Four of 10 control mice died spontaneously before

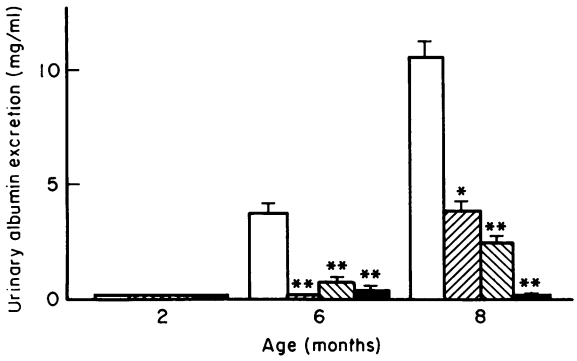

Fig. 1. Effect of PGI_2 (beraprost) on urine albumin excretion. Urine from 5–10 mice in each group was pooled, and albumin concentration in the urine was measured by single radial immunodiffusion (SRID) as described in Materials and Methods. The bars are mean ± s.d. from triplicate determinations. Urine albumin at 2 months was not detectable in this assay (less than 30 μg/ml) in any of four groups. Values for urine albumin excretion at 6 and 8 months were markedly decreased in PGI_2-treated mice compared with controls. $*P < 0{\cdot}01$ *versus* controls; $**P < 0{\cdot}001$ *versus* controls; □, Controls; ▨, 0·5 mg/kg PGI_2; ▧, 1.0 mg/kg PGI_2;, ■, 5·0 mg/kg PGI_2.

reaching this age. By contrast, all 30 mice in the beraprost-treated group were alive throughout the test period until killed.

Microscopically, the glomeruli in controls had the severe mesangial hypercellularity and matrix expansion of their characteristic nephritis (Fig. 2a) [1–3]. Although the degree of glomerular hypercellularity found in 0·5 mg beraprost/kg (61 ± 24 cells/glomerulus) and 1·0 mg beraprost/kg-treated mice (63 ± 22 cells/glomerulus) resembled that in controls (66 ± 12 cells/glomerulus) (Table 1), mice treated with 5·0 mg beraprost/kg showed little or no renal alteration (Fig. 2b). In fact, the number of cells per glomerulus in this group was significantly lower (33 ± 4 cells/glomerulus, $P < 0{\cdot}001$) than in the control group (66 ± 12 cells/glomerulus) (Table 1).

After immunofluorescent staining, all glomeruli from controls showed prominent deposits of IgG, IgM, IgA and C3 (Fig. 3a) in the mesangium and peripheral capillary loops. Beraprost-treated mice showed no obvious difference in glomerular IgG, IgM and IgA deposits compared with controls, as shown in Table 1. However, 5·0 mg beraprost/kg treatment drastically reduced the degree of C3 deposition in glomeruli (Fig. 3b and Table 1). In further study, we examined glomerular deposition of the retroviral envelope glycoprotein, gp70, which is thought to be one of the nephritogenic antigens in NZB/W mice [18,19]. In control mice at 8 months, gp 70 was

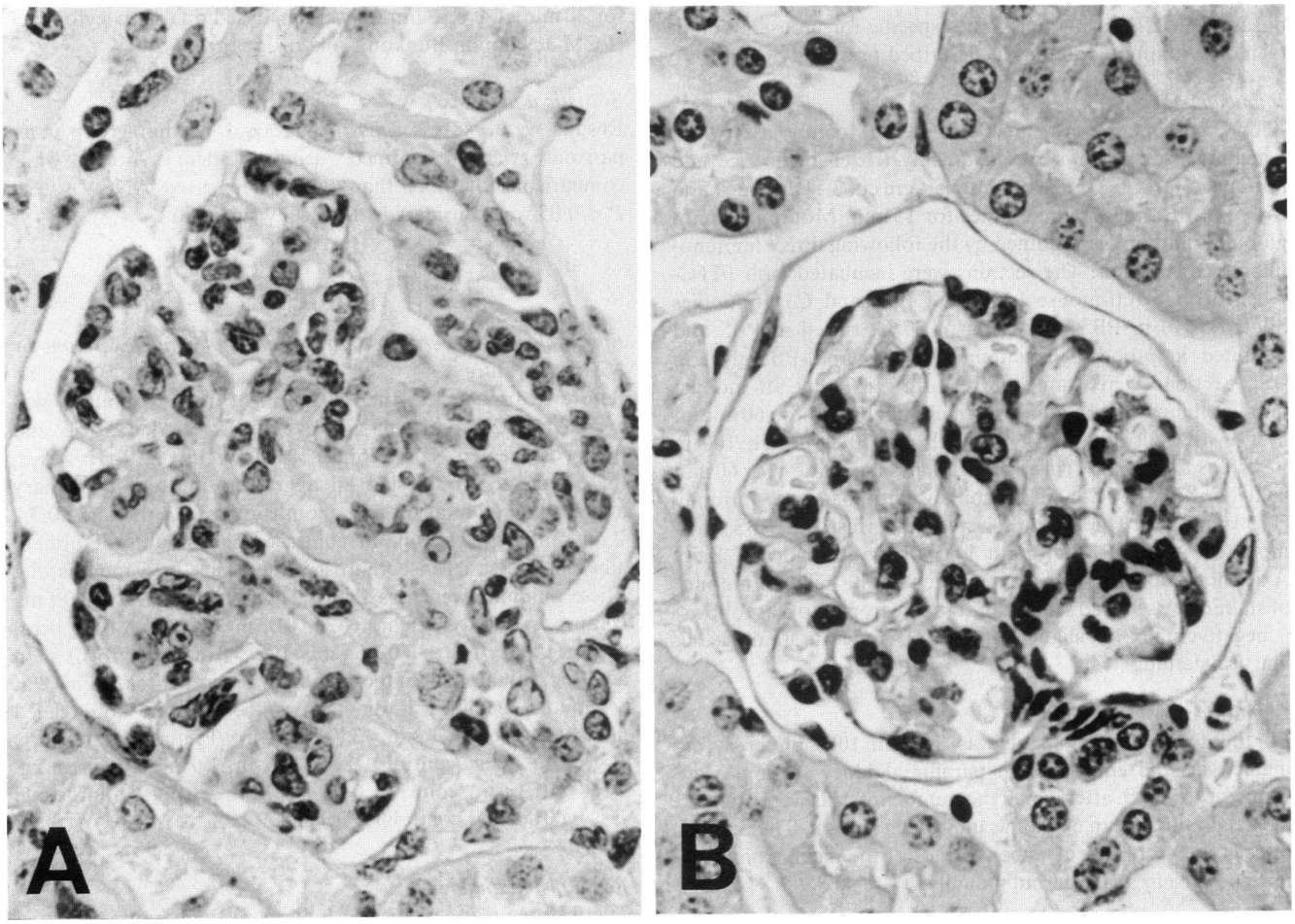

Fig. 2. Light microscopic findings in glomeruli of 8-month-old mice. Glomeruli from control mice showed severe hypercellularity (A) compared with those in beraprost (PGI_2)-treated mice (5 mg/kg) (B). (×200).

Table 1. Histopathological findings in the glomeruli of beraprost (PGI_2)-treated and control NZB/W mice at 8 months of age

Treatment	Cells per glomerulus*	Glomerular staining†				
		IgG	IgM	IgA	C3	gp70
Controls	66 ± 12	3·0	3·0	2·0	2·4	2·2
PGI_2 (mg/kg)						
0·5	61 ± 24	2·4	1·8	2·2	1·8	1·6
1·0	63 ± 22	2·6	2·6	2·4	2·6	1·4
5·0	33 ± 4‡	2·4	2·4	1·4	1·2	0·6

*Cells per glomerulus were counted in more than 50 glomeruli per section under light microscopy. Values are means ± s.d. of the mean from 6–10 mice per group analysed individually.

†The results are expressed as the fluorescent intensity as scored from 0 to 3. The mean score from 6–10 mice per group is shown.

‡$P < 0.001$ *versus* controls.

clearly visible in glomeruli at locations similar to those of C3 deposition (Fig. 4a). By contrast, 5·0 mg beraprost/kg-treated mice had markedly less glomerular gp70 (Fig. 4b), although 0·5 mg and 1·0 mg beraprost/kg treatments had little effect on gp70 deposition in glomeruli of NZB/W mice at 8 months (Table 1).

Immune modulation by PGI_2-treatment of NZB/W F_1 mice

To identify the mechanisms by which PG reduces immune complex deposition in glomeruli, we investigated the effects of beraprost on the humoral and cellular immunity of NZB/W mice. The concentrations of IgG, IgM and IgA in sera were compared in control- and beraprost-treated mice at 2, 6 and 8

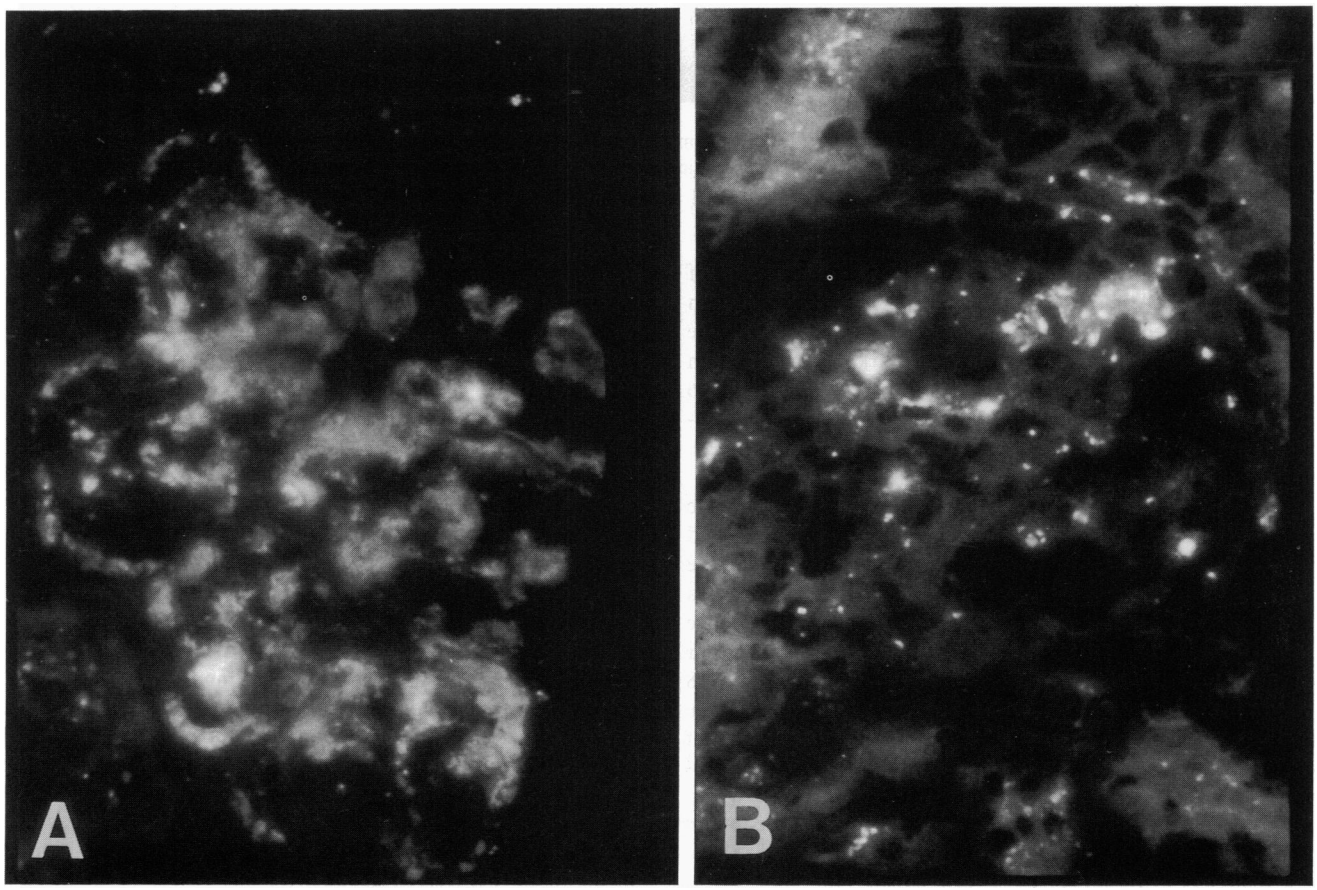

Fig. 3. Glomerular C3 deposition in 8-month-old mice. Glomeruli of controls (A) showed strong C3 staining in mesangial areas and peripheral loops. However, NZB/W mice treated with 5 mg beraprost (PGI_2)/kg (B) had a remarkably reduced degree of glomerular C3 deposits. (×1000).

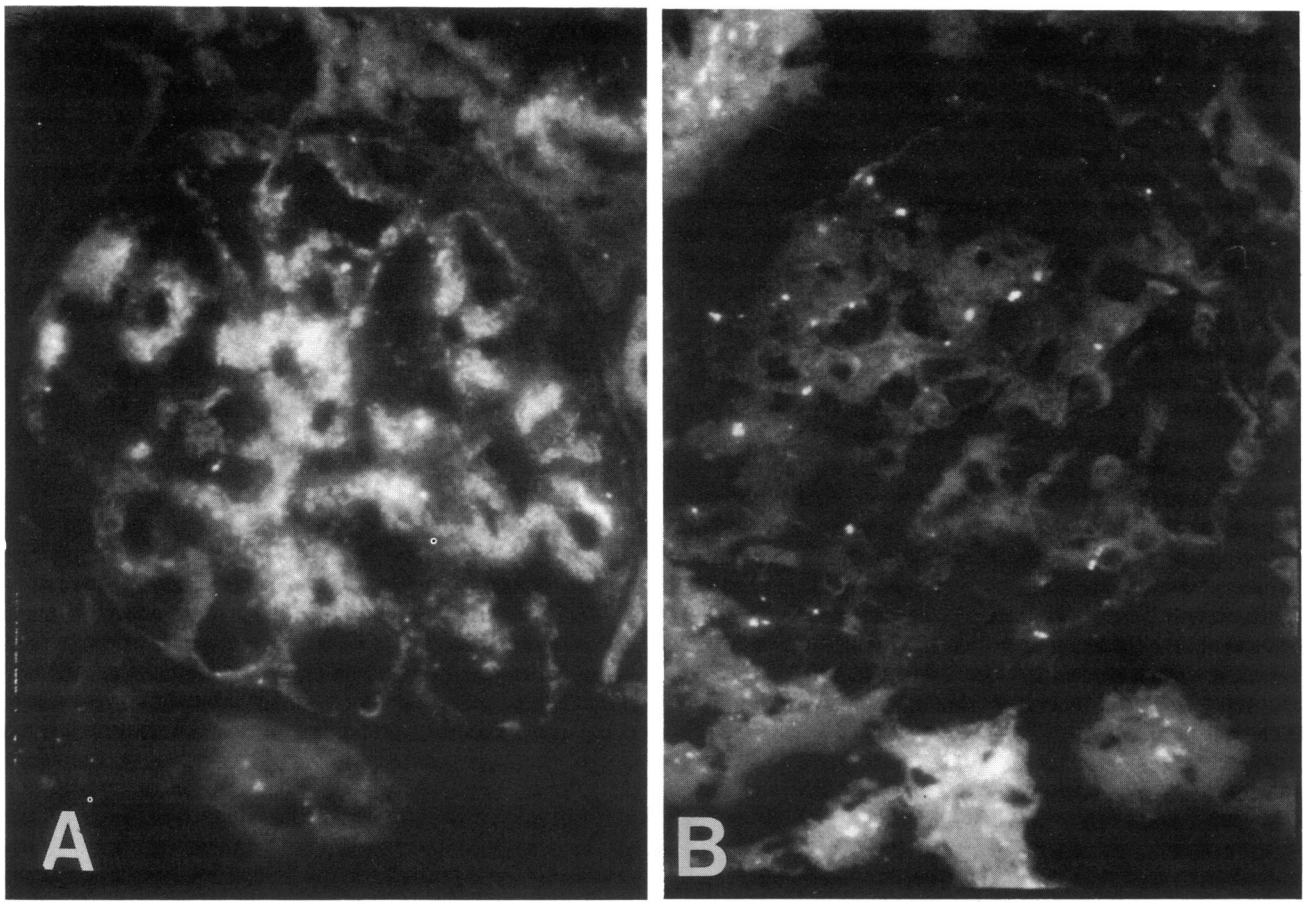

Fig. 4. Retroviral glycoprotein (gp) 70 deposits in 8-month-old mice. Strongly positive mesangial staining was detected in glomeruli of controls (A). In contrast, beraprost (PGI_2)-treated NZB/W mice (5 mg/kg) showed virtually no staining for gp70 in glomeruli (B). (×1000)

months old. As shown in Table 2, beraprost reduced levels of IgG, IgM and IgA in a dose-dependent manner compared with levels in untreated controls at 6 and 8 months old. Similarly, the levels of antibodies to dsDNA were markedly reduced in beraprost-treated mice compared with those of controls at 6 and 8 months, which was particularly notable since anti-dsDNA antibody increases quantitatively as the animals age, as previously described [3,5,19] (Table 3).

Subsequently, the number of helper ($L3T4^+$) and cytotoxic ($Lyt\text{-}2^+$) T cells in splenocytes was compared in control- and beraprost-treated (5 mg/kg) mice. As shown in Fig. 5, the number of $L3T4^+$ cells in 8-month-old controls showed a marked increase compared with that in 2-month-old mice ($30{\cdot}0 \pm 2{\cdot}9 \times 10^6$ per spleen *versus* $20{\cdot}0 \pm 0{\cdot}3 \times 10^6$ per spleen; $P < 0{\cdot}05$), whereas the number of $Lyt\text{-}2^+$cells at 8 months closely resembled that at 2 months old ($11{\cdot}0 \pm 1{\cdot}1 \times 10^6$ per spleen *versus* $12{\cdot}0 \pm 0{\cdot}3 \times 10^6$ per spleen; not significant). On the other hand, in recipients of 5 mg beraprost/kg, by 8 months old total spleen cell number showed a marked decrease compared with that in 8-month-old controls ($36 \pm 9{\cdot}0 \times 10^6$ per spleen *versus* $102 \pm 19 \times 10^6$ per spleen; $P < 0{\cdot}001$). Furthermore, in T cell subset, the number of $L3T4^+$cells decreased to $11{\cdot}6 \pm 1{\cdot}1 \times 10^6$ per spleen, compared with $30{\cdot}0 \pm 2{\cdot}9 \times 10^6$ per spleen in controls ($P < 0{\cdot}001$), and the number of $Lyt\text{-}2^+$ cells was only $6{\cdot}4 \pm 0{\cdot}7 \times 10^6$ per spleen, compared with $11{\cdot}0 \pm 1{\cdot}1 \times 10^6$ per spleen in controls ($P < 0{\cdot}05$).

DISCUSSION

Our present study demonstrated that the stable PGI_2 analogue, beraprost, markedly reduced the amount of albumin excreted in the urine of 6- and 8-month-old NZB/W mice, diminished their mesangial hypercellularity, and decreased the deposition of both C3 and gp70 in their glomeruli compared with untreated controls.

Although 0·5 mg and 1·0 mg beraprost/kg body weight produced no significant renal histological changes when 8-month-old treated mice and untreated controls were compared, the glomeruli from mice given 5 mg beraprost/kg had a striking decrease in mesangial hypercellularity associated with a reduction in renal immune complex deposition (Table 1). In this study we did not examine the effect of beraprost on the lifespan of NZB/W mice, because all our mice were killed at 8 months to obtain renal tissues. However, 4/10 control mice died spontaneously before reaching this age. By contrast, all 30 mice in the beraprost-treated group were alive throughout the test period until killed. Previous studies by Zurier *et al.* [4] and Kelley *et al.* [5] showed that PGE_1 increased the lifespan of NZB/W mice. Kelley *et al.* also demonstrated that PGE_1

Table 2. Immunoglobulin values in beraprost (PGI_2)-treated and control NZB/W mice

	Immunoglobulins (mg/dl)*								
	IgG			IgM			IgA		
Treatment†	2 months	6 months	8 months	2 months	6 months	8 months	2 months	6 months	8 months
Controls	733 ± 11	686 ± 57	826 ± 161	25·3 ± 4·0	58·0 ± 3·5	96·7 ± 5·8	105 ± 10	133 ± 11	195 ± 7
PGI_2 (mg/kg)									
0·5	753 ± 41	593 ± 46	933 ± 116	25·3 ± 4·0	58·7 ± 4·2	76·7 ± 5·8‡	110 ± 14	133 ± 11	210 ± 84
1·0	706 ± 23	566 ± 46‡	693 ± 184	25·3 ± 4·0	48·7 ± 4·6‡	71·0 ± 2·3§	103 ± 12	105 ± 30	115 ± 7§
5·0	727 ± 11	520 ± 34‡	352 ± 110‡	26·7 ± 3·5	33·3 ± 4·6§	66·7 ± 1·5§	106 ± 8	103 ± 12‡	105 ± 21§

* Values represent means ± s.d. as determined by a single radial immunodiffusion method, as described in Materials and Methods.
† Each treated group contained 10 mice.
‡ $P < 0.05$ *versus* controls.
§ $P < 0.01$ *versus* controls.

significantly delayed and reduced the rate of immune complex deposition in the glomeruli of NZB/W mice. Recently, Clark *et al.* [6] found that iloprost, another PGI_2 analogue, significantly delayed the onset of proteinuria and lowered the mortality rate of NZB/W mice, just as PGE_1 had. However, the iloprost, at a dosage of 10 μg twice daily, did not reduce renal immune complex deposition. Our present study suggests that small doses of beraprost, i.e. < 1 mg beraprost/kg body weight, may reduce urine albumin excretion without affecting other nephritic changes in the kidney, the same outcome that Clark *et al.* [6] described after using iloprost. We also demonstrated that the decrease in renal changes attributed to treatment with 5 mg beraprost/kg correlated with a reduction in immune complex deposition in glomeruli of NZB/W mice.

Several mechanisms have been proposed by which PGE_1 and PGI_2 could attenuate renal disease in NZB/W mice. Both PGs are vasodilators; this property could alter renal haemodynamics and change the rate at which circulating immune complexes deposit in the glomeruli [12,13]. Furthermore, PGE_1 and PGI_2 can inhibit the activities of platelets [8,9] and the release of inflammatory mediators derived from platelets, polymorphonuclear leucocytes, macrophages/monocytes and lymphocytes [20,21]. Alternatively, PGE_1 may modulate the immune response, i.e. PGE_1 preserves the T cell mitogenic response, and thereby reduces the amount of circulating immune complexes in humans or murine models with lupus nephritis [5,7,22]. No such effect of PGI_2 on the immune response *in vivo* has been documented.

In our present study we expected beraprost to reduce the concentrations of all immunoglobulins in serum, and to diminish the age-related increase in anti-dsDNA antibody levels in NZB/W mice. Izui *et al.* [19] reported that PGE_1 may prevent the formation of gp70 immune complexes without altering serum anti-dsDNA antibody levels in NZB/W mice. They concluded that PGE may inhibit the immune response to this endogenous viral antigen and suppress the formation of nephritogenic immune complexes such as those containing gp70, without affecting the production of anti-dsDNA antibodies in these mice. In our study, it was not clear that beraprost's reduction of gp70 deposition in glomeruli of NZB/W mice correlated with suppression of gp70 immune

Table 3. Anti-dsDNA antibody values in beraprost (PGI_2)-treated and control NZB/W mice*

	Anti-dsDNA Ab (OD at 492 nm)		
Treatment	2 months	6 months	8 months
Controls	0·425 ± 0·059	1·130 ± 0·129	1·264 ± 0·057
PGI_2 (mg/kg)			
0·5	0·413 ± 0·064	0·735 ± 0·077†	0·945 ± 0·042†
1·0	0·415 ± 0·030	0·634 ± 0·037†	0·782 ± 0·086†
5·0	0·422 ± 0·012	0·658 ± 0·058†	0·595 ± 0·017†

* Values of anti-dsDNA antibody in pooled sera from 6–10 mice in each group were determined by ELISA as described in Materials and Methods. The data are mean OD ± s.d. from triplicate determinations.
† $P < 0.001$ *versus* controls.

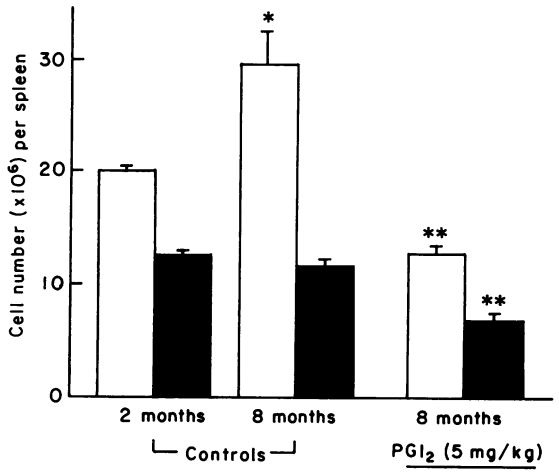

Fig. 5. T cell subset of splenocytes in beraprost (PGI_2)-treated and control NZB/W mice. Numbers of L3T4 (□) or Lyt-2-positive cells (■) per spleen were calculated by staining with MoAbs and analysing by flow cytometry (FCM-1; Nippon Bunkou) as described in Materials and Methods. The bars are the average values from five mice in each group (mean ± s.d.). *$P < 0.05$ *versus* controls at 2 months; **$P < 0.01$ *versus* controls at 8 months.

complex formation in circulating blood. Furthermore, we cannot exclude the possibility that beraprost may also alter renal haemodynamics and prevent immune complex deposition [12,13], or inhibit proliferation of mesangial cells, thus reducing the intake of circulating immune complexes [23,24].

Several investigators have demonstrated that PGE_1 and PGI_2 play a role in regulation of T cell function primed by antigen [25–27]. In 8-month-old NZB/W mice, prior treatment 5 mg beraprost/kg markedly reduced the number of T cells present, especially helper ($L3T4^+$) T cells. In a previous study, treatment of NZB/W mice with anti-L3T4 antibody dramatically reduced anti-dsDNA antibody concentrations, retarded renal disease and prolonged life [28]. That report also suggested that helper ($L3T4^+$) T cells played an important role in the pathogenesis of autoimmunity in NZB/W mice. Our results extend these observations by indicating that treatment of NZB/W mice with a high dose of beraprost may reduce the number of helper ($L3T4^+$) T cells, decrease the synthesis of autoantibodies such as anti-dsDNA antibody, and thereby lessen deposition of immune complexes into glomeruli. To clarify that beraprost can suppress the proliferation and/or activation of helper ($L3T4^+$) T cells, we are now investigating the *in vitro* effect of beraprost on proliferation of lymphocytes or production of various lymphokines in NZB/W mice.

Thus, because the stable prostacyclin analogue, beraprost, ameliorated glomerular injury in nephritic NZB/W mice, we believe that this agent would be useful for the treatment of humans with SLE.

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REFERENCES

1 Dubois EL, Horowitz RE, Demopoulos HB, Teplitz R. NZB/W mice as a model of systemic lupus erythematosus. JAMA 1966; **195**:285–9.
2 Howie JB, Helyer BJ. The immunology and pathology of NZB mice. Adv Immunol 1968; **9**:215–66.
3 Lambert PH, Dixon FJ. Pathogenesis of the glomerulonephritis of NZB mice. J Exp Med 1968; **127**:507–22.
4 Zurier RB, Sayadoff DM, Torrey SB, Rothfield NF. Prostaglandin E_1 treatment of NZB/W mice. I. Prolonged survival of female mice. Arthritis Rheum 1977; **20**:723–8.
5 Kelley VE, Winkelstein A, Izui S. Effect of prostagandin E on immune complex nephritis in NZB/W mice. Lab Invest 1979; **41**:531–7.
6 Clark WF, Parbtani A, McDonald JW, Taylor N, Reid BD, Kreeft J. The effects of a thromboxane synthase inhibitor, a prostacyclin analogue and PGE_1 on the nephritis of the NZB/W F_1 mouse. Clin Nephrol 1987; **28**:288–94.
7 Lin C-Y. Improvement in steroid and immunosuppressive drug resistant lupus nephritis by intravenous prostaglandin E_1 therapy. Nephron 1990; **55**:258–64.
8 Poelstra K, Brouwer E, Baller JFW, Hardonk MJ, Bakker WW. Attenuation of anti-Thy1 glomerulonephritis in the rat by anti-inflammatory platelet-inhibiting agents. Am J Pathol 1993; **142**:441–50.
9 Radomski MW, Palmer RMJ, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br J Pharmacol 1987; **92**:639–46.
10 Boxer LA, Allen JM, Schmidt TM, Yoder M, Baehner RL. Inhibition of polymorphonuclear leucocyte adhesion by prostacyclin. J Lab Clin Med 1980; **95**:672–8.
11 Haurand M, Flohe L. Leukotriene formation by human polymorphonuclear leucocytes from endogenous arachidonate: physiological triggers and modulation by prostanoids. Biochem Pharmacol 1987; **38**:2129–37.
12 Gerber JC, Nies AS. The haemodynamic effects of prostaglandins in the rats. Evidence for important species variation in renovascular responses. Circ Res 1979; **44**:406–10.
13 Gerber JG, Payne NA, Murphy RC, Nies AS. Prostacyclin produced by the pregnant uterus in the dog may act as a circulating vasopressor substance. J Clin Invest 1981; **67**:632–6.
14 Yumura W, Sugimoto N, Nagasawa R, Kubo S, Hirokawa K, Maruyama N. Age-associated changes in renal glomeruli of mice. Exp Gerontol 1989; **24**:237–49.
15 Hara I, Izui S, Dixon FJ. Murine serum glycoprotein gp70 behaves as an acute phase reactant. J Exp Med 1982; **155**:345–57.
16 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965; **2**:235–54.
17 Platt JL, Michael AF. Retardation of fading and enhancement of intensity of immunofluorescence by *p*-phenylenediamine. J Histochem Cytochem 1983; **31**:840–2.
18 Yoshiki T, Mellors RC, Strand M, August JT. The viral envelope glycoprotein of murine leukemia virus and pathogenesis of immune complex glomerulonephritis of New Zealand mice. J Exp Med 1974; **140**:1011–27.
19 Izui S, Kelley VE, McConahey PJ, Dixon FJ. Selective suppression of retroviral gp70–anti-gp70 immune complex formation by prostaglandin E_1 in murine systemic lupus erythematosus. J Exp Med 1980; **152**:1645–8.
20 Kunkel SL, Chensue SW. The role of arachidonic acid metabolites in mononuclear phagocytic cell interactions. Int J Dermatol 1986; **25**:83–89.
21 Marcinkiewicz J, Chain BM. Differential cytokine regulation by eicosanoids in T cells primed by contact sensitization with TNP. Cell Immunol 1993; **149**:303–14.
22 McLeish KR, Gohara AF, Stelzer GT, Wallace JH. Treatment of murine immune complex glomerulonephritis with prostaglandin E_2: dose-response of immune complex deposition, antibody synthesis, and glomerular damage. Clin Immunol Immunopathol 1983; **26**:18–23.
23 Mene P, antibodyboud HE, Dunn MJ. Regulation of human mesangial cell growth in culture by thromboxane A2 and prostacyclin. Kidney Int 1990; **38**:232–9.
24 Stahl RAK, Thaiss F, Haberstroh U, Kahf S, Shaw A, Schoeppe W. Cyclooxygenase inhibition enhances rat interleukin 1β-induced growth of rat mesangial cells in culture. Am J Physiol 1990; **259**:F419–F424.
25 Goodwin JS, Messner RP, Peake GT. Prostaglandin suppression of mitogen-stimulated lymphocytes *in vitro*. Changes with mitogen dose and preincubation. J Clin Invest 1978; **62**:753–60.
26 Burchiel SW: PGI_2 and PGD_2 effects on cyclic AMP and human T cell mitogenesis. Prostaglandins Med 1979; **3**:315–20.
27 McLeish KR, Dyer RD, Senitzer D. Suppression of murine T cell mitogenesis by metabolic products of arachidonic acid. J Immunopharmacol 1982; **4**:53–64.
28 Wofsy D, Seaman WE. Successful treatment of autoimmunity in NZB/NZW F_1 mice with monoclonal antibody to L3T4. J Exp Med 1985; **161**:378–91.