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Effect of FK 506 in the Prophylaxis of Autoimmune Glomerulonephritis in NZB/W_{F1} Mice

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THE F_1 hybrid of New Zealand Black (NZB) and White (NZW) mice spontaneously develops a severe autoimmune disease similar to systemic lupus erythematosus (SLE) in humans.[1] The severe glomerulonephritis kills 50% of these animals at 10 months of age, and 98% at 1 year.

The formation of anti-DNA antibodies and the deposition of immune complexes of G and β_{1C}-globulins, plus DNA and complement along the capillary walls and the mesangia, represent the immunological events that induce the glomerulonephritis.

Several studies have demonstrated the efficacy of immunosuppressive drug therapy in the treatment of the glomerulonephritis in NZB/W_{F1} mice. Cyclophosphamide, azathioprine, and steroids have been reported to be effective when initiated at 5 months of age, but to be of little benefit when used as a short-term prophylaxis in very young mice (1 month) or in mice with advanced renal disease (8 months).[2] Cyclosporine (CyA) has been shown to ameliorate the glomerulonephritis in 8-month-old NZB/W_{F1}.[3] In this study, FK 506, a novel potent immunosuppressive agent with a similar mode of action to CyA, has been used as a prophylactic treatment in 6-week-old female NZB/W_{F1} mice.

MATERIALS AND METHODS

Animals

Female NZB/W_{F1} mice were obtained from the Charles River Co (Japan) and maintained at the animal facility of the University of Pittsburgh.

FK 506

FK 506, donated by Fujisawa Pharmaceutical Co (Osaka, Japan), was suspended in saline and inoculated subcutaneously.

CyA

CyA (Sandoz, Hanover, NJ) was dissolved in intralipid and inoculated subcutaneously.

Experimental Design

Animals were divided into three groups: group 1, mice received no treatment; group 2, 6-week-old mice were treated with FK 506 at a dose of 1.5 mg/kg per day for 12 weeks; and group 3, 6-week-old mice were treated with CyA at a dose of 30 mg/kg per day for 12 weeks.

The animals were periodically killed at the ages of 6 weeks, and 6, 8, and 10 months to obtain samples for investigation.

Anti-DNA Autoantibodies

Antinuclear antibodies (ANA) were measured using an immunofluorescence method. Principle: DNA antibodies in the test sample bind to antigen in the substrate (*Crithidia lucidae*, which contains nDNA); excess serum is removed from the substrate by washing. Fluorescein-conjugated (FITC) antiserum is added to the substrate to which the bound antibodies are attached. After a second wash to remove excess conjugate, the substrate is coverslipped and viewed for fluorescence reaction with the aid of a fluorescent microscope. Only single, well-defined organisms are read.

Con A Lymphocyte Transformation

Splenic mononuclear cells from individual animals were collected and washed after lysis of erythrocytes with Tris-NH_4 Cl, pH 7.2. Cells were washed twice before resuspending in RPMI-1640

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0041-1345/91/$3.00/+0

Table 1. Levels of BUN and Creatinine in Untreated and FK 506- or CyA-treated NZB/W_{F1} Mice

		6-Week-Old	6-Month-Old	8-Month-Old	10-Month-Old
Creatinine (mg/dL)	No treatment	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.9 ± 0.5
	FK 506		0.5 ± 0.1	0.5 ± 0	0.8 ± 0.4
	CyA		0.6 ± 0.2	0.6 ± 0.1	ND
BUN (mg/dL)	No treatment	20.0 ± 2.6	32.5 ± 14.5	40.3 ± 39.2	185.5 ± 194.2
	FK 506		24.8 ± 2.5	21.5 ± 5.0	105 ± 90.1
	CyA		39.0 ± 9.5	28.5 ± 3.7	ND

Data given in mean ± SD.
ND = no difference.

Table 2. Number of Anti-DNA Antibody-Positive Animals in Untreated and FK 506- or CyA-Treated NZB/W_{F1} Mice

	6-Week-Old	6-Month-Old	8-Month-Old	10-Month-Old
No treatment	1/3 (33.3%)	2/4 (50.0%)	3/4 (75.0%)	3/3 (100%)
FK 506		2/4 (50.0%)	2/4 (50.0%)	1/3 (25.0%)
CyA		0/4 (0%)	1/4 (25.0%)	ND

ND = no difference.

(Gibco, Grand Island, NY), supplemented with 10% FBS (Gibco). Cells (5×10^5/well) were incubated with different concentrations of Con A for 3 days at 37°C, 5% co_2, in air. One microcurie [^{3}H]Tdr was added to each well for 24 hours before cell harvesting, and the degree of cell proliferation was assessed by degree of thymidine incorporation.

Histology

After sacrifice and autopsy, kidneys were fixed in formalin and stained by hematoxylin-eosin.

RESULTS

BUN and Creatinine

No significant difference was observed between the three groups (Table 1).

ANA

The effect of FK 506 and CyA on anti-DNA antibodies is shown in Table 2. In the FK 506 group, the incidence of ANA at 8 and 10 months was lower than the other two groups; a further reduction in the incidence of ANA antibodies was observed in the CyA group.

Con A Stimulation

As shown in Fig 1, there was a gradual decrease in the lymphocyte-proliferating ability following stimulation with Con A from 6- to 10-month-old animals. No difference in this response was observed between control animals and FK 506-treated animals.

Histology

No evidence of glomerulonephritis was observed in the kidneys of 6-week-old NZB/W_{F1} mice. At 6 months of age, the histology showed the onset of disease in all the groups. At 8 and 10 months of age, no differences were observed between the different groups (Fig 2) and histological evaluation showed enlarged glomeruli, with proliferation of all glomerular cellular elements, membranous thickenings of the glomerular capillary walls, and focal capillary occlusion with proteinaceous deposit; lymphocyte infiltration was also evident.

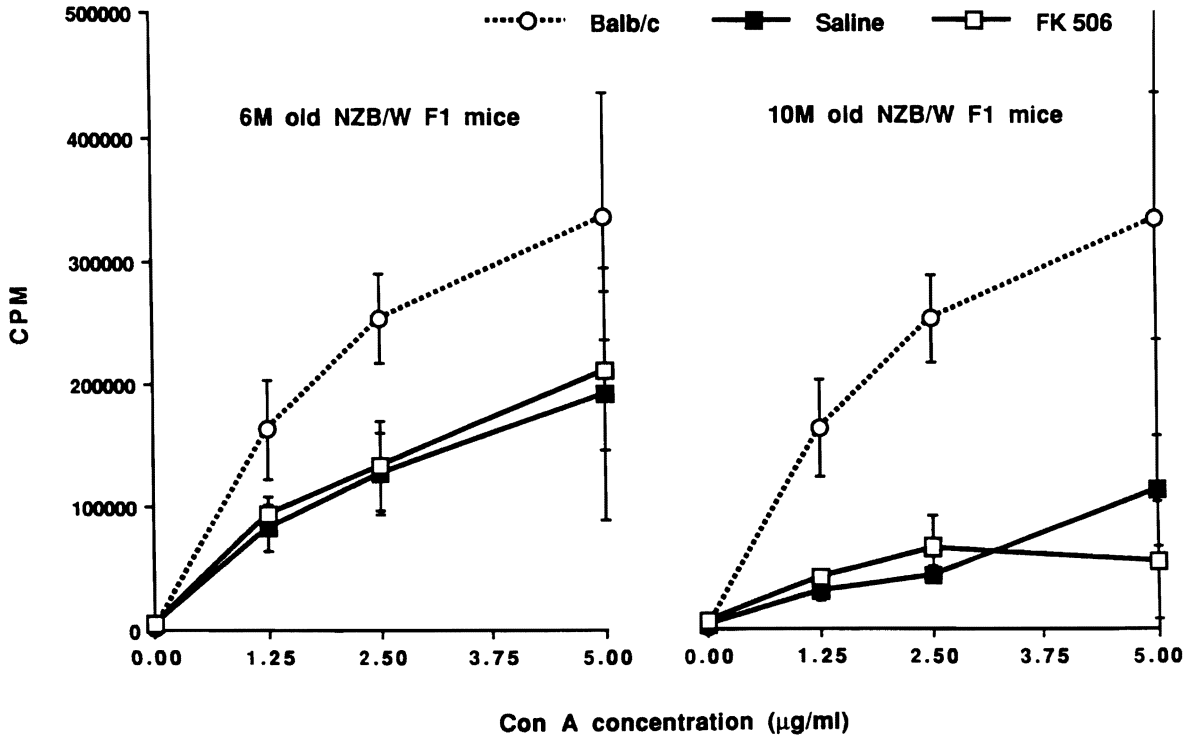

Fig 1. Proliferation of splenic MNC from BALB/c, untreated, and FK 506-treated NZB/W_{F1} mice at 6 and 10 months of age, following stimulation with different concentrations of Con A.

DISCUSSION

The glomerulonephritis in NZB/W_{F1} mice is reported to be related to an immunological event characterized by production of anti-DNA antibodies and immune complex deposition. Several immunosuppressive agents like CyA, cyclophosphamide, and azathioprine have been shown to be effective in the treatment of the disease. The data from this study show that prophylaxis with FK 506 did not prevent or ameliorate the histological changes of the glomerulonephritis. However, a lower incidence of ANA was observed in the treated animals. Borel et al[4] postulated a direct correlation between ANA levels and glomerulonephritis, but Okudaira et al[3] reported, in 1987, that the beneficial effect of CyA on the disease was not associated with a decreased level of ANA. Our data also showed the absence of a relationship between ANA levels and disease evolution.

In this study, FK 506, a novel potent immunosuppressive agent, which suppresses T-cell immunity, lowered the incidence of ANA antibodies but did not ameliorate histopathological or clinical evidence of the disease. Treatment with FK 506 also did not affect the substantially reduced lymphocyte response to Con A (seen in NZB/W_{F1}) compared with normal animals, implying that the defective T-cell reactivity was not modified by this FK 506 regimen. This is consistent with the histological and clinical observations. Different drug doses and different treatment periods will be required in future attempts to prevent glomerulonephritis in this particular model of lupus disease.

REFERENCES

1. Howie JR, Helyer BJ: Adv Immunol 9:215, 1968

2. Steinberg AD, Gelfand MC, Hardin JA, et al: Arthritis Rheum 18:9, 1975

3. Okudaira H, Terada E, Okudaira K: Animal Models: Assessing the Scope of Their use in Biomedical Research. New York, AR Liss, 1987, p 157

4. Borel Y, Lewis RM, Andre-Schwartz J, et al: J Clin Invest 61:276, 1978