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# Retinoic Acid Reduces Autoimmune Renal Injury and Increases Survival in NZB/W $F_1$ Mice

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**Retinoic acids, a group of natural and synthetic vitamin A derivatives, have potent antiproliferative and anti-inflammatory properties. Recently, retinoic acids were reported to inhibit Th1 cytokine production. We investigated the effects of retinoic acid on lupus nephritis in a model of NZB/NZW $F_1$ (NZB/W $F_1$) mice. Three-month-old NZB/W $F_1$ mice were separated into two groups: one treated with all-*trans*-retinoic acid (ATRA; 0.5 mg i.p., three times weekly for 7 mo) and one with saline as a control. Compared with controls, ATRA-treated mice survived longer and exhibited a significant reduction of proteinuria, renal pathological findings including glomerular IgG deposits, and serum anti-DNA Abs. Splenomegaly was less marked in the treated mice than in controls. Transcripts encoding IFN-$\gamma$, IL-2, and IL-10 in splenic $CD4^+$ T cells were significantly reduced in treated mice compared with controls. We conclude that treatment with ATRA in SLE-prone NZB/W $F_1$ mice significantly alleviates autoimmune renal disorder and prolongs survival; this may thus represent a novel approach to the treatment of patients with lupus nephritis.** ***The Journal of Immunology*****, 2003, 170: 5793–5798.**

New Zealand Black/White $F_1$ mice develop a systemic autoimmune disease characterized by immune complex-type glomerulonephritis (lupus nephritis), vasculitis, lymphadenopathy, and splenomegaly, closely resembling human systemic lupus erythematosus (SLE)[2] (1–3). Much of the pathology of this condition is mediated by autoantibodies and autoreactive lymphocytes (4, 5). In both patients with SLE and murine models, treatment with immunosuppressive agents such as corticosteroids or cyclophosphamide can reduce the progression of lupus nephritis and prolong survival (6–9). However, these agents have severe potential side effects, including increased susceptibility to infections (10–12) and the development of malignant tumors (13). Therefore, the development of new drugs with few side effects is needed.

Early studies linked the functional imbalance between Th1 and Th2 cells to the pathogenesis of autoimmune disease (14, 15). In addition, several studies have described the predominance of Th2 cytokines, including IL-4, -6, and -10, in NZB/W $F_1$ mice and other lupus-prone mice (16, 17). More recently, Th1 cytokines, including IFN-$\gamma$ and IL-2, have been reported to elicit autoimmune tissue destruction in lupus-prone mouse strains (18–20).

Retinoic acids (RA), a group of natural and synthetic derivatives of vitamin A, play important regulatory roles in cellular proliferation and differentiation. In vitamin A-deficient mice, RA shift the immune response from a Th2-type to a Th1-type response (21). Although RA have some side effects, such as teratogenicity, weight loss, bone fracture, anemia, and liver damage, RA are currently used to treat acute promyelocytic leukemia (22) and inflammatory diseases such as psoriasis (23), acne (24), and rheumatoid arthritis (25). In the present study to investigate whether all-*trans*-retinoic acid (ATRA) would be beneficial in the treatment of SLE, we assessed the effect of ATRA on the development of lupus nephritis in NZB/W $F_1$ mice. Our data provide evidence that ATRA treatment significantly alleviates autoimmune renal disorder and prolongs survival.

## Materials and Methods

### *Mice*

NZB/W $F_1$ mice were purchased from Japan SLC (Shizuoka, Japan) and maintained in our specific pathogen-free animal facility.

### *ATRA treatments and clinical and laboratory tests*

ATRA was obtained from Sigma-Aldrich (St. Louis, MO). Three-month-old NZB/W $F_1$ mice were divided into two groups and i.p. injected with 0.5 mg of ATRA dissolved in 0.5 ml of saline or with 0.5 ml of saline alone (controls). Injections were repeated three times weekly for 5–7 mo.

Two sets of experiments were conducted; one (eight experimental and control mice each) was for examination of survival rate, cumulative incidence of proteinuria, and long term toxicity of ATRA treatment, and the other for examination of splenomegaly, pathology, immunopathology of the kidney, serum and message levels of cytokines, and proteinuria. In the latter, experiments were terminated when the mice reached 8 mo of age. Mice were sacrificed under ether anesthesia. Dying mice, which usually exhibited proteinuria of 1000 mg/dl or more, were sacrificed before 8 mo of age. Urinary protein levels were assessed semiquantitatively using albumin reagent strips (Hema-Combistix; Bayer/Sankyo, Tokyo, Japan) on a monthly basis. Grades of proteinuria were expressed as 0 (none), 1 (30–99 mg/dl), 2 (100–299 mg/dl), 3 (300–999 mg/dl), or 4 (1000 or more mg/dl). Mice exhibiting grade 2 or greater proteinuria were regarded as positive. When mice became positive for proteinuria, tests were repeated. Serum samples were obtained by cardiac puncture. To evaluate the side effects of ATRA treatment, we examined weight loss, skin lesions, bone fracture, anemia, and liver damage in mice at 3 and 10 mo of age, before and after initiation of ATRA treatment, respectively. Briefly, anemia was analyzed by measuring the hematocrit of freshly collected blood. Liver function was analyzed by measuring serum aspartate aminotransferase and alanine aminotransferase using an autoanalyzer (model 917; Hitachi, Tokyo, Japan).

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[2] Abbreviations used in this paper: SLE, systemic lupus erythematosus; ALP, alkaline phosphatase; ATRA, all-*trans*-retinoic acid; gcs, glomerular cross-sections; M$\phi$, macrophages; RA, retinoic acid.



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### *Renal histopathology and immunopathology*

Kidney tissues were either snap-frozen in OCT compound (Miles Scientific, Naperville, IL) for cryostat sectioning or fixed in 10% neutral-buffered formalin. Formalin-fixed tissues were embedded in paraffin, 4-$\mu$m-thick sections were stained with periodic acid-Schiff, and glomerular, tubular, vascular, and perivascular pathologies were evaluated morphometrically by light microscopy (26). The glomerular pathology of 50 glomerular cross-sections (gcs)/kidney was scored on a semiquantitative scale: 0, normal (35–40 cells/gcs); 1, few lesions with slight proliferative change and mild hypercellularity (41–50 cells/gcs); 2, moderate hypercellularity (51–60 cells/gcs), segmental and/or diffuse proliferative change, hyalinosis, and moderate exudates; and 3, severe hypercellularity (>60 cells/gcs) with segmental or global sclerosis and/or severe necrosis, crescent formation, and heavy exudation. Tubular pathology was evaluated by counting the percentage of tubules exhibiting damage (dilation, atrophy, or necrosis) among 200 randomly selected tubules. Perivascular inflammatory cell infiltration was evaluated by scoring the number of cell layers surrounding 10 randomly chosen inter- and intralobular arteries (0, none; 1, <5 layers surrounding less than half the vessel; 2, 5–10 layers surrounding more than half the vessel; 3, >10 layers surrounding more than half the vessel). To examine IgG deposits within renal glomeruli, kidney cryostat sections (4 $\mu$m thick) were stained with FITC-conjugated goat anti-mouse IgG, IgG1, IgG2a, IgG2b, or IgG3 Ab (Serotec, Oxford, U.K.). Fluorescence intensity within glomerular capillary walls was scored on a scale of 0–3 (0, none; 1, weak; 2, moderate; 3, strong). At least 10 glomeruli/section were analyzed. Scoring was performed by two investigators using coded slides.

Cryostat sections of kidney tissues were also stained using an immunoperoxidase method for T cells with anti-CD4 and anti-CD8, for B cells with anti-B220 rat anti-mouse mAbs (BD PharMingen, San Diego, CA), and for macrophages (M$\phi$) with F4/80 hybridoma culture supernatant (HB198; American Type Culture Collection, Manassas, VA). As a specificity control, normal rat IgG was used as the primary rat Ab. Numbers of T cells, B cells, and M$\phi$ within the kidney were expressed as number of cells per glomerulus or number of cells per field, as described (26). Scoring was performed by two investigators using coded slides.

### *Anti-DNA Abs*

Ig class-specific anti-DNA Abs were measured by ELISA as previously described (27). Each well of flat-bottom, 96-well plates (Costar, Cambridge, MA) was coated with calf thymus DNA (Sigma-Aldrich), dried at 37°C, and washed in isotonic PBS (pH 7.4). Nonspecific protein binding was blocked by coating the plate with 10% goat serum in PBS. After 100 $\mu$l of each serum sample appropriately diluted with PBS was added, and the preparation was incubated for 2 h at 37°C, each well was washed and allowed to react with 100 $\mu$l of appropriately diluted alkaline phosphatase (ALP)-conjugated goat anti-mouse Ig, IgG1, IgG2a, IgG2b, or IgG3 Abs (ICN Biomedical, Costa Mesa, CA) for 2 h at 37°C. ALP activity was measured using an ALP substrate kit (Wako Pure Chemical, Osaka, Japan) containing phenyl phosphate and 4-aminoantipyrine. Pooled sera from 8-mo-old NZB/W $F_1$ mice were used as standards. Titers of the standard sera were defined as follows: for total IgG, 1000 U/ml; IgG1, 170 U/ml; IgG2a, 340 U/ml; IgG2b, 220 U/ml; and IgG3, 270 U/ml. Absorbance at 490 nm for each well was measured by a microplate reader (Benchmark; Bio-Rad, Richmond, CA). Ab titers were determined from absorbances using a standard curve constructed for each IgG subclass.

### *Isolation of $CD4^+$ T lymphocytes*

Soon after RBC were removed by hemolysis using distilled water, spleen cells were passed through a column containing nylon wool fiber (Wako Pure Chemical), and the passed cells were used as T cells. The percentage of $CD3^+$ cells in these cells was 88% as determined by flow cytometry. $CD4^+$ T cells were isolated from the general T cell population by positive selection using biotinylated anti-CD4 mAb (RM4-5; BD PharMingen) with streptavidin-labeled magnetic beads (M-280; Dynal, Oslo, Norway) and a magnet (Dynal). The purity of the $CD4^+$ T cells was >94%.

### *Measurements of mRNA transcripts and serum levels of Th1 and Th2 cytokines*

To examine levels of expression of Th1 (IL-2 and IFN-$\gamma$) and Th2 (IL-4 and IL-10) cytokines in $CD4^+$ T cells, mRNA transcripts in $CD4^+$ T cells isolated from ATRA-treated and control NZB/W $F_1$ mice were semiquantitatively measured using the RT-PCR method. Total RNA was extracted from the $CD4^+$ T cells using Isogen (Nippon Gene, Tokyo, Japan). The RT reaction was performed with RNA using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY). The resulting RT product was used as a cDNA template for PCR analysis, which was performed using primers for IL-2, IFN-$\gamma$, IL-4, and IL-10 (Stratagene, La Jolla, CA) and the PCR Gene Amp System 9600 (PerkinElmer, Foster City, CA). The expression of a housekeeping gene, $\beta$-tubulin, was detected as a 400-bp PCR product using specific oligonucleotide primers (28) as an internal standard for semiquantitative analysis of the PCR amplification. The level of expression of each cytokine was designated as a ratio of cytokine to $\beta$-tubulin mRNA determined by photodensitometry (ATTO, Tokyo, Japan). Serum IL-2, IFN-$\gamma$, IL-4, and IL-10 levels were measured with an ELISA kit (BD PharMingen, San Diego, CA) following the manufacturer's instructions.

### *Statistics*

Data were analyzed using the Kruskal-Wallis test for comparing survival curves, and the Mann-Whitney *U* test for comparing group means. A value of $p < 0.05$ by two-tailed *t* test was considered significant.

## Results

### *Survival and proteinuria*

NZB/W $F_1$ mice treated with ATRA survived significantly longer than control mice ($p < 0.05$). Seven months after the initiation of treatment (mice aged 10 mo), as shown in Fig. 1*A*, while only three of eight control NZB/W $F_1$ mice (37.5%) that had been treated with saline alone were alive, six of eight mice (75%) treated with ATRA survived. Fig. 1*B* shows the cumulative incidence of proteinuria, in which NZB/W $F_1$ mice in the control group began to develop proteinuria at 4 mo of age, and the cumulative incidence reached 75% at 10 mo of age, while the incidence (12.5%) in mice treated with ATRA was significantly lower than that in the control group at this age. As shown in Fig. 1*C*, urinary protein levels in mice treated with ATRA were significantly reduced compared with those in controls (grades of proteinuria at 8 mo of age, $1.1 \pm 0.3$ vs $3.1 \pm 0.4$, respectively; $n = 8$; $p < 0.001$). Two mice in the ATRA-treated group died before 10 mo of age, one at 8 mo and one at 9 mo of age. The cause of death in the former case was unknown. (There was no evidence of renal failure, bone fracture, or liver failure; however, infection could not be excluded.) The latter exhibited proteinuria before death, and there were histopathological changes in the kidney noted on postmortem exhibited. All five dead mice in the control group exhibited severe proteinuria and renal pathology.

### *Renal pathology*

Common histologic characteristics of NZB/W $F_1$ mice include the development of severe glomerulonephritis with proliferative changes in mesangial and endothelial cells of glomeruli, capillary basement membrane thickening, and chronic obliterative changes, i.e., mononuclear cell infiltrates in interstitium, glomerular IgG deposits, and vasculopathy (primarily degenerative, occasionally inflammatory). Fig. 2 compares representative renal pathological findings between the two groups of mice. NZB/W $F_1$ mice treated with saline at 8 mo of age exhibited glomerular hypercellularity, hyalinosis, segmental or global sclerosis, and crescent formation; damage in 28% of tubules; and abundant leukocytes surrounding vessels. In contrast, renal glomeruli, tubules, and vasculature in NZB/W $F_1$ mice treated with ATRA remained almost intact ($n = 8$; $p < 0.01$–$0.005$; Fig. 3*A*). In NZB/W $F_1$ mice treated with saline alone at 5–8 mo of age, there were numerous kidney-infiltrating T cells ($CD4^+$ and $CD8^+$), B cells, and M$\phi$ in periglomerular, interstitial, and perivascular areas. In contrast, these infiltrates were significantly less marked in mice treated with ATRA ($n = 8$; $p < 0.01$–$0.005$; Fig. 3*B*).

### *Ig deposition in the kidney*

Table I shows the extent of glomerular deposition of IgG (total IgG, IgG1, IgG2a, IgG2b, and IgG3) in the two groups of mice at 5–8 mo of age. Compared with the findings in controls, glomerular

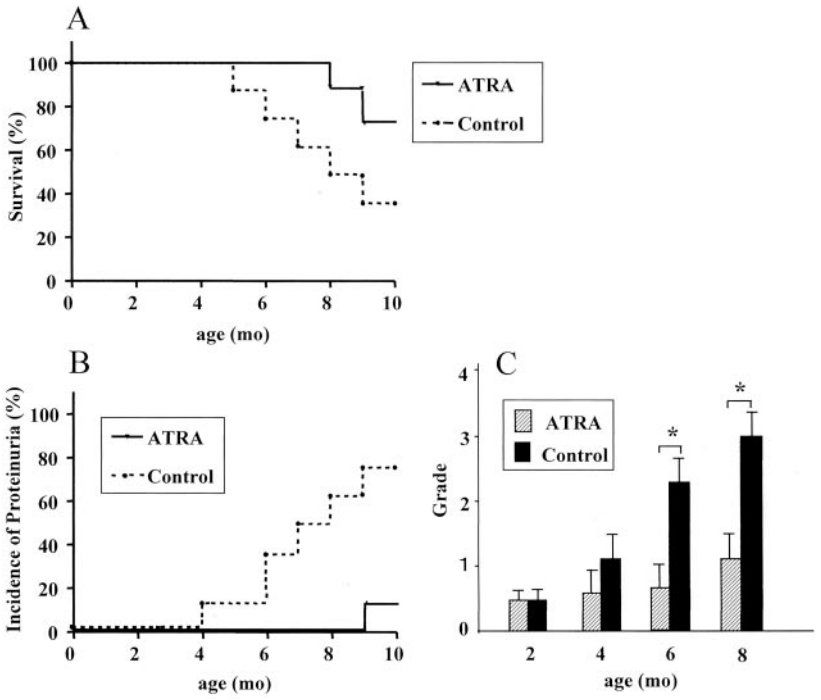


**FIGURE 1.** Comparisons of percent survival, cumulative incidences of proteinuria, and urinary protein levels between eight NZB/W $F_1$ mice treated with ATRA and eight control mice. *A*, Percent survival. The difference is significant ($p < 0.05$). Values are the mean ± SD. *B*, Cumulative incidence of proteinuria ($p < 0.001$). C, Urinary protein levels (∗, $p < 0.001$).

deposits of total IgG, IgG2a, and IgG2b in mice treated with ATRA were significantly less marked ($p < 0.05$). Notably, however, no significant difference was observed in the deposition of IgG1 and IgG3 between the two groups.

*Anti-DNA Abs*

Elevated anti-DNA Ab levels are characteristic of NZB/W $F_1$ mice. To determine whether ATRA treatment alters the anti-DNA Ab isotype profile in NZB/W $F_1$ mice, we evaluated serum anti-DNA Ab isotype (total IgG, IgG1, IgG2a, IgG2b, and IgG3) concentrations in the two groups of NZB/W $F_1$ mice (Fig. 4). Total IgG and IgG2a anti-DNA Ab concentrations were significantly lower in NZB/W $F_1$ mice treated with ATRA than in those treated with saline ($n = 8$; $p < 0.05$ and $p < 0.05$, respectively). In contrast, no significant difference was observed in concentrations of IgG1, IgG2b, and IgG3 anti-DNA Abs between the two groups.

*Splenomegaly*

Splenomegaly, a hallmark of disease in NZB/W $F_1$ mice, was diminished in NZB/W $F_1$ mice treated with ATRA; the mean spleen weight in NZB/W $F_1$ mice treated with ATRA was half that in NZB/W $F_1$ mice treated with saline (0.18 ± 0.04 g vs 0.36 ± 0.08 g, respectively; $n = 8$; $p < 0.03$).

*Cytokine mRNA expression in $CD4^+$ T cells*

To determine whether ATRA alters cytokine expression in $CD4^+$ T cells in spleen, we compared amounts of Th1 and Th2 cytokine transcripts in $CD4^+$ T cells between the two groups of NZB/W $F_1$ mice at 5–8 mo of age (Fig. 5). Levels of expression of IL-2, IFN-γ, and IL-10 mRNA in NZB/W $F_1$ mice treated with ATRA were significantly lower than those in NZB/W $F_1$ mice treated with

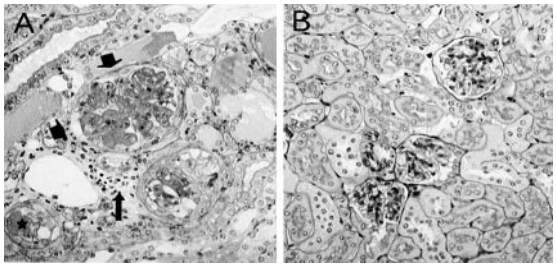


**FIGURE 2.** Histopathological findings for kidney. *A*, A kidney from a control NZB/W $F_1$ mouse at 8 mo of age. Note sclerotic glomeruli (short, wide arrow, grade 3), tubular damage and cast (star), and perivascular leukocytic infiltrates (long arrow, grade 2). *B*, A kidney from a mouse treated with ATRA at a comparable age. Periodic acid-Schiff hematoxylin stain; magnification, ×400.

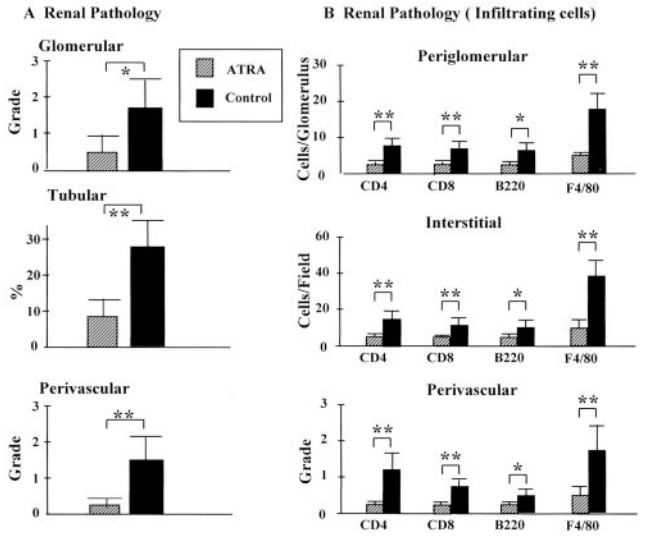


**FIGURE 3.** Differences in histopathological scores of renal disease and extent of immune cell infiltrates between NZB/W $F_1$ mice treated with ATRA and controls. *A*, Scores of glomerular and tubular damage and perivascular inflammatory cell infiltrates ($n = 8$; ∗, $p < 0.01$; ∗∗, $p < 0.005$). *B*, Numbers of infiltrates of T cells, B cells, and Mϕ in the kidneys ($n = 8$; ∗, $p < 0.01$; ∗∗, $p < 0.005$). Values are the mean ± SD.

Table I. *Comparisons of fluorescence intensities of glomerular deposits of IgG in NZB/W $F_1$ mice*[a]

| | Control | ATRA-Treated |
|---|---|---|
| Total IgG | 2.5 ± 0.2 | 1.8 ± 0.3[b] |
| IgG1 | 2.1 ± 0.3 | 1.5 ± 0.7 |
| IgG2a | 2.5 ± 0.4 | 1.7 ± 0.7[b] |
| IgG2b | 2.1 ± 0.4 | 1.1 ± 0.5[b] |
| IgG3 | 1.3 ± 0.3 | 1.0 ± 0.4 |

[a] Fluorescence intensity was scored on a scale of 0–3 (0, none; 1, weak; 2, moderate; 3, strong; $n = 8$. Values are the mean ± SD.
[b] $p < 0.05$.

saline ($p < 0.02$, $p < 0.005$, and $p < 0.02$, respectively). However, the difference in expression of IL-4 mRNA was not significant. These data were comparable to those for serum IL2, IFN-γ, IL-4, and IL-10 levels, as measured by ELISA (Table II). While serum IL-4 levels did not differ significantly between the two groups of mice, serum IL-2, IFN-γ, and IL-10 levels were significantly lower in ATRA-treated mice than in controls.

### *Side effects*

During the experimental period there was no evidence of side effects in mice treated with ATRA. The mean body weight in treated mice did not differ significantly from that in controls at 10 mo of age (46.7 ± 4.3 vs 48.4 ± 5.2 g). As shown in Table III, there were no signs of anemia, liver dysfunction, skin lesions, or bone fractures in treated mice.

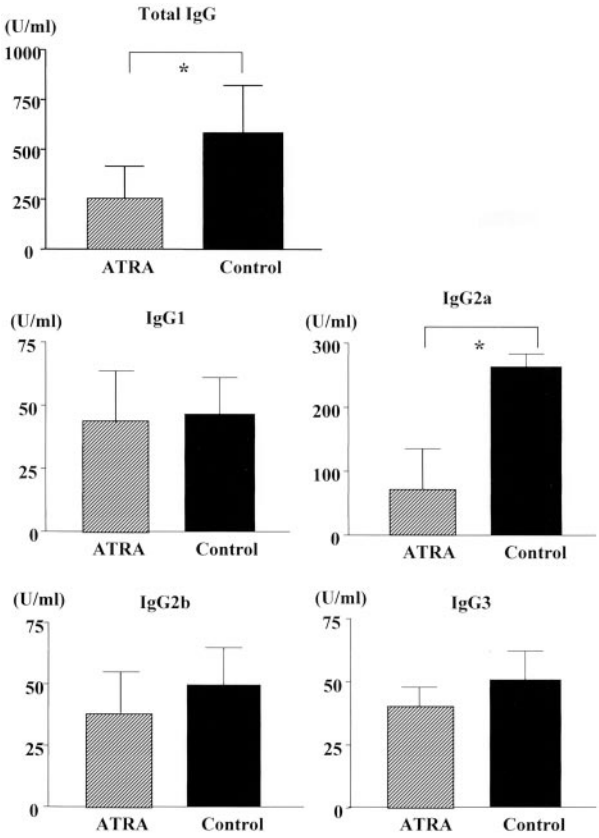


**FIGURE 4.** Comparisons of serum levels of anti-DNA Abs of different IgG subclasses between NZB/W $F_1$ mice treated with ATRA and controls ($n = 8$; ∗, $p < 0.05$). Values are the mean ± SD.

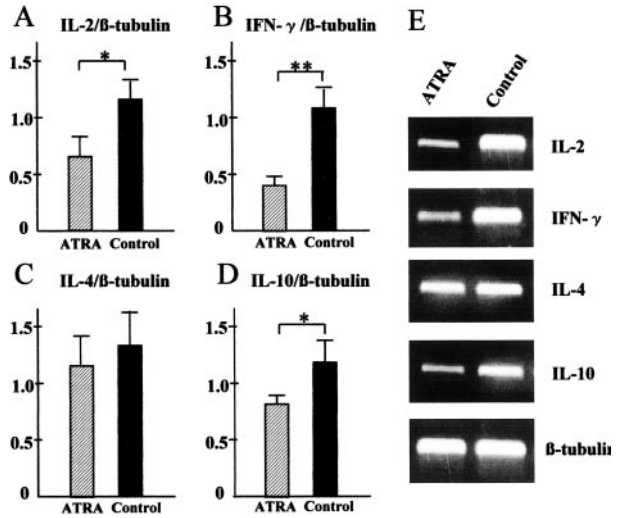


**FIGURE 5.** Reduction of IL-2, IFN-γ, and IL-10 transcription in $CD4^+$ T cells from ATRA-treated NZB/W $F_1$ mice. Transcript levels for IL-2, IFN-γ, IL-4, and IL-10 were assessed by RT-PCR in $CD4^+$ T cells from NZB/W $F_1$ mice treated with ATRA and controls. *A*, IL-2 ($n = 8$; ∗, $p < 0.02$). *B*, IFN-γ ($n = 8$; ∗, $p < 0.05$). *C*, IL-4 ($n = 8$; not significantly different). *D*, IL-10 ($n = 8$; ∗, $p < 0.02$). Values are the mean ± SD. *E*, Representative results for expression of mRNA of IL-2, IFN-γ, IL-4, and IL-10.

## Discussion

We have provided evidence that ATRA treatment markedly alleviates autoimmune tissue injuries and prolongs survival in a lupus model of NZB/W $F_1$ mice. The treatment had wide effects on multiple pathologic components of SLE in these mice, including renal pathology, glomerular IgG deposits, leukocytic infiltrates, elevated cytokine levels (IFN-γ, IL-2, and IL-10), and elevated serum levels of anti-DNA Abs. ATRA may thus be beneficial for the treatment of SLE and possibly for other systemic autoimmune diseases.

Previous studies have demonstrated that retinoic acids inhibit Th1 cytokine production by T cells (29–31). In the present study we found that ATRA treatment of NZB/W $F_1$ mice significantly reduced levels of the Th1 cytokines IFN-γ and IL-2 produced by CD4 T cells, with a greater effect on IFN-γ. Serum levels of anti-DNA Abs of the IgG2a isotype, isotype switching of which is known to be dependent on Th1 cytokines (32), were significantly decreased in these mice by treatment with ATRA in association with a reduction of glomerular deposits of IgG2a. Although levels of circulating and glomerular deposited IgG3 are associated with glomerulonephritis in the MRL-*Fas*$^{lpr}$ mouse model of SLE (33), complement-fixing IgG2a anti-DNA Abs are thought to be predominant nephritogenic autoantibodies in NZB/W $F_1$ mice (34).

Table II. *Treatment with ATRA reduces IL-2, IFN-γ, and IL-10, but not IL-4, in sera of NZB/W $F_1$ mice*[a]

| Cytokines (pg/ml) | ATRA-Treated Mice | Control Mice |
|---|---|---|
| IL-2 | 2.6 ± 1.4 | 5.3 ± 1.5[b] |
| IFN-γ | 0 ± 0 | 210 ± 60[c] |
| IL-4 | 12.6 ± 11.0 | 17.1 ± 14.3 |
| IL-10 | 111 ± 33 | 184 ± 40[b] |

[a] Sera were obtained from NZB/W $F_1$ mice treated with ATRA or saline at 5–8 mo of age. IL-2, IFN-γ, IL-4, and IL-10 were measured by ELISA. Values are the mean ± SD ($n = 8$). ATRA vs control.
[b] $p < 0.005$, ATRA vs control (by Mann-Whitney *U* test).
[c] $p < 0.001$, ATRA vs control (by Mann-Whitney *U* test).

Table III. *Tests for long term toxicity of ATRA treatment in NZB/W $F_1$ mice*

| ATRA-Treated Mice | *n* | Body Weight (g) | Anemia Ht (%) | AST (IU/liter) | ALT (IU/liter) | Skin Lesions | Bone Fracture |
|---|---|---|---|---|---|---|---|
| 3 mo of age | 8 | 28.7 ± 1.2 | 48.4 ± 1.5 | 67.4 ± 9.5 | 32.8 ± 10.8 | − | − |
| 10 mo of age | 8 | 46.7 ± 4.3 | 47.6 ± 1.9 | 75.5 ± 10.4 | 35.3 ± 13.6 | − | − |
| Significance (*p* value) | | | NS | NS | NS | | |

[a] Tests were carried out before (3 mo of age) and after ATRA treatment (10 mo of age). Values are the mean ± SD. Ht, hematocrit; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Data for anemia and liver functions in ATRA-treated mice are not significantly different from those in mice before treatment.

These findings are consistent with the concept that Th1 cytokines, especially IFN-γ, play an important role in the pathogenesis of autoimmune disease in NZB/W $F_1$ mice. IFN-γ has been reported to be a cytokine of central importance in murine lupus by promoting the switch of IgM to IgG2a subclass, leading to glomerular injury, and by inducing apoptosis of renal parenchymal cells (35). For example, administration of IFN-γ deteriorates lupus nephritis in NZB/W $F_1$ mice, and administration of Abs to IFN-γ or of soluble IFN-γ receptors reduces autoantibody levels, diminishes glomerular IgG deposits and lymphocytic infiltrates in the kidney, and prolongs survival in these mice (36). Thus, ATRA appears to predominantly inhibit IFN-γ expression, leading to reduced IgG2a anti-DNA Ab production and IgG2a deposition in glomeruli and resulting in the prevention of fatal autoimmune kidney disease in the NZB/W $F_1$ strain.

There are, however, several findings conflicting with this concept in the present studies. For example, although ATRA affected neither serum nor mRNA transcript levels of Th2 cytokine IL-4, it significantly reduced the levels of IL-10, another Th2 cytokine, in NZB/W $F_1$ mice. IL-10 is a cytokine that accelerates B cell proliferation and differentiation (37). Administration of anti-IL-10 Abs has been reported to delay the onset of autoimmunity in NZB/W $F_1$ mice via inhibition of autoantibody production (38). Thus, the beneficial effects of ATRA on NZB/W $F_1$ disease observed in the present study may be due at least in part to suppression of IL-10 production.

The glomerular deposition of IgG2b, isotype switching of which from IgM is dependent on Th2 cytokines, was also reduced in mice treated with ATRA, although the extent of glomerular deposition of other Th2-dependent IgG1 and IgG3 did not differ between the ATRA-treated and control mice. There is a report demonstrating that many hybridoma clones producing high affinity anti-DNA Abs derived from NZB/W $F_1$ mice belong to IgG2b, a finding suggesting that IgG2b anti-DNA Abs are also nephritogenic in these mice (39). Thus, although it is evident that ATRA treatment causes selective down-regulation of cytokines in NZB/W $F_1$ mice, its effects may not be confined to those on Th1 cytokines. While retinoic acid receptors are expressed in T cells (40), further studies are needed to clarify the subset distribution of retinoic acid receptors and the signaling pathway that influences cytokine regulation.

The mechanisms of the effects of ATRA in alleviating renal disease in NZB/W $F_1$ mice appear to be more complex, since ATRA has strong antiproliferative effects. It has been reported that administration of ATRA can alleviate the glomerular injury induced in rats with anti-Thy1.1 mAb through a mechanism that reduces proliferating cell nuclear Ag-positive cells and growth factors, such as α-smooth muscle actin and platelet-derived growth factor-B (41). In addition, ATRA is known to inhibit inducible NO synthase, which causes oxidative injury in glomerulonephritis (42). In any case, a more thorough understanding of the mechanisms of the effects of ATRA on autoimmune disorders and a more thorough investigation of the long term toxicity of ATRA may provide new approaches to the treatment of patients with SLE and other related systemic autoimmune diseases.

## Acknowledgments

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