Retinoic Acid Reduces Glomerular Injury in a Rat Model of Glomerular Damage

JÜRGEN WAGNER,* CLAUDIUS DECHOW,* CHRISTIAN MORATH,* INGO LEHRKE,* KERSTIN AMANN,‡ RUDIGER WALDHERR,* JÜRGEN FLOEGE,‡ and EBERHARD RITZ*  

Departments of *Nephrology and †Pathology, University of Heidelberg, Heidelberg, Germany, and ¨Department of Nephrology, University of Hannover, Hannover, Germany.

Abstract. In the reaction of kidneys to injury, cytokine-driven proliferation plays an important role and precedes the development of glomerulosclerosis. There is great interest in agents that may interfere with such proliferation. Therefore, a rat model of mesangioproliferative glomerulonephritis (induced by anti-Thy1.1) was studied, and the effects of all-trans-retinoic acid (all-trans-RA) and isotretinoin, powerful antiproliferative and anti-inflammatory substances, on glomerular damage and cell proliferation were examined. Vehicle-injected control rats were compared with rats treated with daily subcutaneous injections of 10 mg/kg body wt all-trans-RA or 40 mg/kg body wt isotretinoin (n = 9 to 11 per group), using either a pretreatment (days −2 through 8) or posttreatment (days +3 through +8) protocol, i.e., starting before or after the induction of anti-Thy1.1 nephritis, respectively. All-trans-RA prevented the BP increase evoked by anti-Thy1.1 (anti-Thy1.1/vehicle, 112.2 ± 4.8 mmHg; anti-Thy1.1/RA, 87.5 ± 2.5 mmHg; P < 0.001). Treatment with all-trans-RA or isotretinoin produced a 70% decrease in the urinary albumin excretion rate (P < 0.02). Periodic acid-Schiff staining of saline-perfused kidneys (day 8) revealed significantly fewer glomerular cells in RA-treated nephritic rats (anti-Thy1.1/vehicle, 97 ± 3.1 cells/glomerulus; anti-Thy1.1/RA, 80 ± 4.4; P < 0.02; control/vehicle, 69 ± 1.2). No difference was observed between all-trans-RA and isotretinoin treatment. The capillary occlusion scores were significantly lower for the anti-Thy1.1/RA-treated group (1.9 ± 0.1) than for the anti-Thy1.1/vehicle-treated group (2.9 ± 0.5, P < 0.001). In the anti-Thy1.1/vehicle-treated group, 11.9 ± 1.1 glomerular cells were proliferating cell nuclear antigen-positive; however, in the anti-Thy1.1/RA-treated group, only 5.3 ± 0.8 cells were proliferating cell nuclear antigen-positive (P < 0.002; control, 2.2 ± 0.2). Glomerular mitoses were reduced by 67% in the anti-Thy1.1/RA-treated group, compared with the anti-Thy1.1/vehicle group (P < 0.002). Glomerular staining for platelet-derived growth factor B-chain was significantly reduced in anti-Thy1.1-treated nephritic rats in the presence of isotretinoin or all-trans-RA, compared with the vehicle-treated group (P < 0.001). It is concluded that all-trans-RA limits glomerular proliferation, glomerular lesions, and albuminuria in an established model of renal damage. The findings point to retinoids as potential novel modulators of glomerular injury.

Cellular proliferation is one of the hallmarks of the renal response to injury. In spontaneous or experimentally induced inflammatory renal diseases (such as mesangioproliferative glomerulonephritis or IgA or lupus nephritis) and in the remnant kidney model, mesangial cell proliferation precedes the development of glomerular scarring and sclerosis (1,2). Glomerular hypercellularity contributes to expansion of the mesangial space, deposition of extracellular matrix proteins, and collapse of glomerular capillaries (3,4).

In the search for substances that interfere with this proliferative process, retinoids, i.e., natural or synthetic derivatives of vitamin A, are of great interest because they are known to exert strong antiproliferative effects in most cell types, such as myocytes, vascular smooth muscle cells, and mesangial cells (5–7). Additionally, they have an anti-inflammatory action and modulate extracellular matrix synthesis in a complex and tissue-specific manner (8). These processes are also relevant for kidney disease.

Retinoic acids (RA) act via RA receptors (RAR) α, β, and γ and retinoid X receptors (RXR) α, β, and γ (9,10). The antiproliferative effects of retinoids are attributable to modulation of gene transcription by these compounds (11–13). RA and synthetic retinoids demonstrate potent anti–activator protein-1 (AP-1) activity and have been proposed as substances that downregulate AP-1-regulated genes. RA interfere with other proinflammatory pathways as well as nuclear factor-κB and creb-binding protein/P300, which integrate a number of cell regulatory and signaling pathways (14–16).

A role for retinoids in kidney differentiation has been demonstrated in double-knockout mice deficient for RAR-α/RXR-α. In tissue culture, retinoids influence glomerular number and tubular differentiation (17–19). Little is known regarding the effects of retinoids on kidneys of mature animals or human subjects (20,21). The antiproliferative effects of

Received June 9, 1999. Accepted December 30, 1999.

Dr. William G. Couser served as Guest Editor and supervised the review and final disposition of this manuscript.

Correspondence to Dr. Jürgen Wagner, Department of Nephrology, University Hospital, University of Heidelberg, Bergheimerstrasse 56a, D-69115 Heidelberg, Germany. Phone: +49 6221 91120; Fax: +49 6221 16 24 76; E-mail: juergen_wagner@med.uni-heidelberg.de

1046-6673/1108-1479

Journal of the American Society of Nephrology

Copyright © 2000 by the American Society of Nephrology
retinoids prompted us to examine whether retinoids reduce mesangial cell proliferation and thus limit glomerular injury in a rat model of mesangiocapillary glomerulonephritis. We studied the effects of all-trans-RA, a prototypic natural retinoid, and of isotretinoin (13-cis-RA), a second generation retinoid that is far less toxic and is used in human therapeutic regimens. Two protocols were used. In the first series, treatment with all-trans-RA was initiated before injection of the anti-Thy1.1 antibody (pretreatment). In the second series, treatment of rats with all-trans-RA or isotretinoin was started on the third day after antibody injection (posttreatment).

Materials and Methods

Experimental Protocol

The rat anti-Thy1.1 model of mesangioproliferative glomerulonephritis was induced by injection of OX-7, a monoclonal antibody against the Thy1.1 antigen (European Collection of Animal Cell Cultures, Salisbury, United Kingdom) (22). Male Wistar rats (180 to 200 g; Charles River, Sulzfeld, Germany) were used. In the first set of experiments (pretreatment), four experimental groups (n = 9/group) were examined. Two groups were treated with daily subcutaneous injections of 10 mg/kg body wt all-trans-RA (Sigma-Aldrich Chemie, Steinheim, Germany) dissolved in arachis oil and 5% DMSO. The other two groups received daily subcutaneous injections of arachis oil and DMSO only (vehicle). One of the groups treated with all-trans-RA and one of the vehicle-injected groups received intravenous injections of 1 mg/kg body wt OX-7 antibody into the tail vein, 2 d after the beginning of treatment. The other two groups received intravenous injections of phosphate-buffered saline (PBS) only (control). The experiment was terminated 8 d after injection of the antibody.

In the second set of experiments, treatment with daily subcutaneous injections of 10 mg/kg body wt all-trans-RA or vehicle began 2 d after intravenous injection of OX-7 or PBS (control) (posttreatment). This set of experiments included two additional experimental groups, which were treated with daily subcutaneous injections of 40 mg/kg body wt isotretinoin (13-cis-RA) (Hoffmann La Roche, Basel, Switzerland) dissolved in arachis oil and 5% DMSO, as described above. One of those groups received the OX-7 antibody and the other received PBS (control) 2 d before treatment with retinoids. In the second set of experiments, renal biopsies were performed on day 4 after antibody injection and animals were euthanized on day 8 after induction of nephritis.

BP was determined on days −3 and +8 by tail-cuff plethysmography, with light ether anesthesia. For euthanasia, animals were given intramuscular injections of xylazine (5 mg/kg body wt; Bayer Vital, Leverkusen, Germany) and ketamine (10%, 100 mg/kg body wt; WDT, Garbsen, Germany). Rats were perfused with saline solution containing 0.5 g/L procaine hydrochloride, at a defined pressure of 110 mmHg, by retrograde insertion of a cannula into the abdominal aorta (23). The inferior vena cava was incised to drain blood. Creatinine clearance was calculated after enzymatic determination (using a creatinine kit; Hoffmann La Roche) of serum and urinary (from 24-h urine samples) creatinine levels, with a Hitachi autoanalyzer (Frankfurt, Germany).

Signs of retinoid toxicity were apparent after treatment with all-trans-RA and included cholelithiasis and some hair loss. Animals treated with all-trans-RA lost approximately 8% of their body weight (217 ± 4.1 g) compared with the anti-Thy1.1/vehicle-treated group (233 ± 3.1 g), whereas no weight loss was observed for the anti-Thy1.1/isotretinoin-treated group (233 ± 4.2 g).

Renal Morphologic Studies

Tissue for light microscopy was fixed in 10% buffered formalin and embedded in paraffin. Sections (4 μm) were stained with the periodic acid-Schiff (PAS) reagent and counterstained with hematoxylin. In the PAS-stained sections, the total number of nuclei in each glomerular cross section was determined, and mesangiolysis was graded semiquantitatively on a scale from 0 to 3+, as described (24). For all morphologic determinations, the investigator was blinded with respect to experimental groups. Accuracy was determined by a second investigator for some parameters. A minimum of 30 cortical glomeruli with diameters of at least 100 μm were examined in each biopsy specimen.

Capillary Occlusion Scores

In PAS-stained sections, the extent of occlusion of the capillary tuft area was determined using a semiquantitative scoring system (0, little or no occlusion; 1+, up to 25% of the total capillary tuft area occluded; 2+, 25 to 50% occluded; 3+, 50 to 75% occluded; 4+, >75% of the capillary tuft area occluded in at least 30 cortical glomeruli).

Free Bowman Space

Morphometric analysis was performed using PAS-stained sections. At least 100 glomeruli were analyzed for each animal. For area measurements, a semiautomated video image-analyzing system (Videoplan; Kontron Co., Eching, Germany) was used (23). At a primary magnification of ×160, the mean area of glomerular profiles was measured. The area included within the Bowman capsule was determined and the total capillary tuft area was subtracted, to calculate the free Bowman space. No significant difference was observed in the area including the Bowman capsule among the different groups.

Total Glomerular Cell Counts

Total glomerular cell counts were determined in at least 30 cortical glomeruli with diameters of at least 100 μm for each kidney, in PAS-stained sections (25). Visible glomerular mitoses (anaphase and metaphase) were counted in 100 glomeruli for each kidney, in PAS-stained sections.

Immunohistochemistry

Saline-perfused slices (4 μm) of renal tissue obtained from comparable renal areas in all rats were fixed in methyl Carnoy’s solution and processed using the direct or indirect immunoperoxidase technique, as described previously (22). The primary antibodies used included those described previously (22,25): 1A4 (an antibody against α-smooth muscle actin [α-SMA]; Dako, Glostrup, Denmark) (26), 19A2 (a murine IgM monoclonal antibody against human proliferating cell nuclear antigen [PCNA]; Coulter, Hialeah, FL), and PGF-007 (a murine monoclonal antibody against the human platelet-derived growth factor [PDGF] B-chain; Mochida Pharmaceutical, Tokyo, Japan).

For all biopsies, negative controls involved substitution of the primary antibody with equivalent concentrations of an irrelevant murine monoclonal antibody or normal rabbit IgG. For each biopsy, more than 30 cross sections of consecutive cortical glomeruli with diameters of at least 100 μm were evaluated, in a blinded manner. Mean values of the number of proliferating (PCNA-positive) cells per biopsy were calculated. For evaluation of immunoperoxidase staining for α-SMA, each glomerulus was graded semiquantitatively, as described (25), and the mean score per biopsy was calculated. The scores reflect changes in the extent but not the intensity of mesangial matrix staining (0, diffuse weak staining; 1+, up to 25% of the glomerular tuft exhibiting focally increased staining; 2+, 25 to 50% of the tuft exhibiting staining; 3+, 50 to 75% of the tuft exhibiting staining; 4+, >75% of the glomerular tuft exhibiting strong staining).

For double-staining of proliferating cells with anti-PCNA and anti-α-SMA antibodies (see above), we used the Super Sensitive ISH De-
tection System (Biogenex Laboratories, San Ramon, CA), according to the recommendations of the manufacturer, with 3,3’-diaminobenzidine chromogen for PCNA and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate for α-SMA. Fibrin deposition was stained according to the method of Masson (27).

Albuminuria

For determination of albumin levels in urine, rats were placed in metabolic cages and urine was collected for 24 h. Albuminuria in rats was determined essentially as described by Magnotti et al. (28), on a 96-well enzyme-linked immunosorbent assay plate, using a peroxidase-conjugated anti-rat albumin antibody (ICN-Biomedical, Eschwege, Germany). All measurements were performed in quadruplicate.

Statistical Analyses

All values are expressed as mean ± SEM, unless stated otherwise. Statistical significance (defined as P < 0.05) was evaluated using the nonparametric Mann–Whitney test.

Results

Effects of RA Treatment on BP and Albuminuria

Systolic BP was significantly elevated in vehicle-treated glomerulonephritic rats on day 8 after induction of nephritis (Figure 1). Treatment with all-trans-RA had no effect on BP in control rats but completely abrogated the BP increase in glomerulonephritic animals (Figure 1). The rate of albumin excretion was markedly increased in vehicle-treated glomerulonephritic rats, compared with controls. However, all-trans-RA produced a 70% reduction of albuminuria in glomerulonephritic rats (Figure 2A). In the second set of experiments, in which treatment with retinoids was initiated on day 3, albu-

Figure 1. All-trans-retinoic acid (all-trans-RA) inhibition of the BP increase in rats with anti-Thy1.1-induced glomerulonephritis. No significant effect of all-trans-RA on BP was observed in control rats. Data are presented as mean ± SEM (n = 9 rats/group). Con, control; Veh, vehicle; **, P < 0.01; ***, P < 0.001.

Figure 2. (A) Reduction of 24-h albumin excretion rate by all-trans-RA in rats with anti-Thy1.1-induced glomerulonephritis (GN). Pretreatment with all-trans-RA attenuated the increase in albumin excretion rates. No change in albuminuria was observed in control rats treated with all-trans-RA. (B) Reduction of 24-h albumin excretion rate by all-trans-RA or isotretinoin with the posttreatment protocol. Data are mean ± SEM (n = 9 animals/group).

minuria was reduced to similar extents by all-trans-RA or isotretinoin (Figure 2B). Creatinine clearance assays demonstrated a significant reduction in renal function in anti-Thy1.1-treated nephritic rats but almost complete recovery in the presence of all-trans-RA (Figure 3).

Effects of RA on Renal Histologic Features

Figure 4 depicts typical PAS-stained sections of glomeruli from animals with anti-Thy1.1-induced glomerulonephritis, after treatment with vehicle (Figure 4A) or all-trans-RA (Figure 4B). Note the reduction in swelling of the glomerular tuft in the all-trans-RA-treated rat. The capillary occlusion score was significantly higher for vehicle-treated rats with anti-Thy1.1-induced glomerulonephritis compared with controls, but was significantly lower with all-trans-RA pretreatment (P < 0.001) (Figure 5A). The number of totally occluded glomeruli was also significantly less in all-trans-RA-treated nephritic rats (11.2 ± 1 out of 50 glomeruli in the anti-Thy1.1/vehicle-treated group versus 1.2 ± 0.2 in the anti-Thy1.1/RA-treated group, P < 0.004). In these animals, the free Bowman space...
Effects of RA on Glomerular Cell Proliferation

The number of visible mitoses in glomerular cells was lower (i.e., glomerular tuft swelling was prevented) by pretreatment with all-trans-RA (Figure 5B). The number of cells per glomerular cross section was significantly increased in vehicle-treated glomerulonephritic rats compared with control rats. Pretreatment with all-trans-RA did not affect glomerular cell counts in control rats but significantly reduced glomerular cell counts in rats with anti-Thy1.1-induced glomerulonephritis, compared with vehicle-treated nephritic rats ($P < 0.01$) (Figure 6A). Similarly, posttreatment with all-trans-RA and isotretinoin decreased glomerular cell numbers (control/vehicle, 69 ± 1.9; anti-Thy1.1/vehicle, 106 ± 2.7; anti-Thy1.1/isotretinoin, 95 ± 2.1; anti-Thy1.1/RA, 92 ± 2.2; $P < 0.02$, compared with the anti-Thy1.1/vehicle-treated group). Mesangiolysis was examined in renal biopsies obtained on day 4 in the posttreatment experiments. On day 4, we did not observe any difference in the extent of mesangiolysis in the nephritic rats treated with either vehicle, isotretinoin, or all-trans-RA (anti-Thy1.1/vehicle, 1.85 ± 0.09; anti-Thy1.1/isotretinoin, 1.8 ± 0.1; anti-Thy1.1/RA, 1.81 ± 0.13; NS). Mesangiolysis was barely detectable in anti-Thy1.1/vehicle-treated rats on day 8.

Effects of RA on Glomerular Cell Proliferation

The number of visible mitoses in glomerular cells was lower in rats pretreated with all-trans-RA compared with vehicle-treated rats with anti-Thy1.1-induced glomerulonephritis ($P < 0.001$) (Figure 6B). Figure 4 depicts typical glomerular staining for PCNA-positive cells in rats with anti-Thy1.1-induced nephritis, without (Panel C) or with (Panel D) all-trans-RA. The number of PCNA-positive cells was markedly increased in nephritic rats compared with control rats, but was significantly less in all-trans-RA-treated rats compared with vehicle-injected rats ($P < 0.001$) (Figure 7). The PCNA-positive glomerular cell number was low in control rats without glomerulonephritis.

The experiments involving posttreatment with all-trans-RA or isotretinoin revealed significantly reduced staining of glomerular cells for α-SMA compared with untreated glomerulonephritic rats (α-SMA scores: anti-Thy1.1/vehicle, 2.6 ± 0.1; anti-Thy1.1/isotretinoin, 2.0 ± 0.3; anti-Thy1.1/RA, 1.83 ± 0.2; both $P < 0.02$ versus anti-Thy1.1/vehicle; control/vehicle, 0.2 ± 0.1). Double-staining for PCNA and α-SMA revealed colocalization of these markers primarily in mesangial cells (Figure 4, E and F). In the posttreatment experiments, glomerular staining for PDGF-B revealed significantly reduced staining in the glomeruli of anti-Thy1.1-treated nephritic animals treated with isotretinoin or all-trans-RA, compared with vehicle-treated rats (Figure 8).

Effects of RA on Monocytes/Macrophages and Glomerular Fibrin Deposition

The number of glomerular macrophages/macrophages was markedly higher in anti-Thy1.1/vehicle-treated rats than in control animals (Figure 9A). Isotretinoin significantly reduced the number of macrophages compared with anti-Thy1.1-treated nephritic rats, whereas reduction in the presence of all-trans-RA did not reach statistical significance. Deposition of glomerular fibrin was enhanced in anti-Thy1.1-treated nephritic rats and was significantly less in anti-Thy1.1/RA-treated rats ($P < 0.001$) (Figure 9B).

Discussion

This study shows that retinoid treatment of rats with experimental mesangio proliferative glomerulonephritis causes a significant reduction in glomerular damage, as indicated by different functional and histologic markers. Albuminuria, a marker of glomerular damage, was reduced by 70% in all-trans-RA- or isotretinoin-treated rats. The decreases in albuminuria were similar for rats pretreated or posttreated with all-trans-RA. All-trans-RA also prevented the BP increase in glomerulonephritic rats that was previously described (22). This effect of all-trans-RA may reflect the lesser extent of glomerular damage in the presence of the retinoid. BP levels were not lowered in the presence of all-trans-RA in control rats. There was no significant difference in the efficacy of isotretinoin compared with all-trans-RA in reducing albuminuria. Glomerular structure was better preserved in the presence of all-trans-RA or isotretinoin, as demonstrated by reductions in capillary occlusion scores, larger free Bowman space areas, and a 61% reduction in total glomerular cell counts compared with vehicle-treated nephritic rats. The comparable efficacies of pretreatment and posttreatment exclude the possibility that the effects of all-trans-RA might be attributable to interference with OX-7 antibody binding or action. With the posttreatment protocol, no differences in mesangiolysis, i.e., the primary lesion in this model of glomerular damage, were observed between the treatment groups. The effects of retinoids in the posttreatment series were even more remarkable because the treatment period lasted only 5 d.

The preservation of glomerular structure by retinoids may be attributable to their antiproliferative action, which is a main characteristic of these compounds. In addition to carcinoma or
HeLa cells, antiproliferative effects of retinoids were observed for all cell types investigated, e.g., vascular smooth muscle cells, cardiac myocytes, and mesangial cells stimulated with fetal calf serum (5–7,13). Inhibition of proliferation is presumably attributable to inhibition of AP-1 transactivation (11,12) as a result of direct protein-protein interactions of retinoid receptors with the AP-1 complex, downregulation of c-Fos and Jun-1, or other mechanisms (11). This effect of retinoids has been examined only in cell culture experiments, but it may explain the inhibition of genes that are involved in glomerular damage and act via AP-1, such as angiotensin II, PDGF, and the endothelins (5,29,30). Angiotensin II-induced growth pro-

Figure 4. (A and B) Typical periodic acid-Schiff staining of glomeruli of anti-Thy1.1-treated nephritic rats treated with vehicle (A) or all-trans-RA (B). Note the reduction of glomerular tuft swelling in all-trans-RA-treated glomeruli. (C and D) Representative sample of immunohistochemical staining for proliferating cell nuclear antigen (PCNA)-positive glomerular cells in glomeruli of anti-Thy1.1-treated nephritic rats treated with vehicle (C) or all-trans-RA (D). PCNA-positive cells were undetectable in nonnephritic control rats. (E and F) Representative sample of immunohistochemical double-staining for PCNA (brown) and α-smooth muscle actin (red) in anti-Thy1.1-treated nephritic rats treated with vehicle (E) or all-trans-RA (F).
motion in vascular smooth muscle cells can be blocked by all-trans-RA (29). Similarly, retinoids block the cell hypertrophy induced by endothelin-1 in embryonic cardiac myocytes (5). PDGF-B was shown to be inhibited by retinoids during mouse embryogenesis (30). In this study, glomerular staining for PDGF-B was less intense after treatment with either all-trans-RA or isotretinoin. In this model, PDGF may serve as a marker of proliferation. This notion is in agreement with the finding that fewer cells express PCNA in the presence of retinoids. It is unlikely that the antiproliferative action of retinoids is mediated only by inhibition of the AP-1 complex. These compounds influence protooncogenes, such as c-myc and the H-ras pathway (31), and compete for c-erb-B-binding protein/P-300, an integrator complex that integrates a number of signaling pathways. The antiproliferative function of retinoids has led to their use in the treatment of cancer and hyperplastic skin diseases (32–34).

All-trans-RA is used for human patients for the treatment of leukemias or prostate or metastatic lung disease, but even low doses may cause vitamin A-like toxicity. Isotretinoin, a second-generation retinoid, is much less toxic and is used in human patients for the treatment of severe acne (35). The purpose of our study was to establish whether retinoids can

Figure 5. (A) Significant reduction of capillary occlusion scores by pretreatment with all-trans-RA in rats with anti-Thy1.1-induced glomerulonephritis. The scores for control rats were not affected. (B) Preservation of the free Bowman space by pretreatment with all-trans-RA in rats with anti-Thy1.1-induced glomerulonephritis. Data are mean ± SEM (n = 9).

Figure 6. (A) All-trans-RA reduction of total glomerular cell counts in rats with anti-Thy1.1-induced glomerulonephritis (pretreatment protocol). Cell numbers were significantly elevated in vehicle-treated nephritic rats compared with controls. (B) All-trans-RA reduction of the frequency of glomerular mitoses in rats with anti-Thy1.1-induced glomerulonephritis.
influence renal damage in nephritis. We therefore selected high doses of all-trans-RA and isotretinoin, which exceeded dose recommendations for human subjects (rat toxicity data were kindly provided by Hoffmann LaRoche, courtesy of Dr. M. Klaus, in a personal communication). We observed weight loss (−27%) for all-trans-RA-treated rats, with some hair loss, cheilitis, and keratitis, but animals treated with isotretinoin demonstrated no signs of toxicity.

All-trans-RA is known to interact specifically with the RAR. However, in tissues and organs, natural retinoids may isomerize into different retinoid forms, which may interact with RAR or RXR in a tissue-specific manner. Therefore, the experiments described above do not permit conclusions regarding which receptors in the kidney are responsible for the observed retinoid effects. RAR were demonstrated to mediate retinoid- and vitamin A-specific effects but may be differentially expressed during development (9,11). In contrast to RAR, RXR form heterodimers with other members of the steroid receptor superfamily, enabling cross-talk with the vitamin D receptor, peroxisome-proliferative activator receptor, thyroid receptor, or other receptors (36,37). Little is known regarding the expression of these receptors in the kidney. In renal failure, impairment of the interaction between vitamin D and RXR, because of downregulation of RXR, was observed (37). During ontogeny, double-knockout mice deficient for RAR isotypes show kidney agenesis or aplasia (17). Vitamin A has teratogenic effects involving renal development (38). Retinoids control tubular and glomerular development in metanephros cultures (39). These observations indicate that retinoids are closely involved in renal development. Our findings support the notion that these vitamin A derivatives may also play a role in kidney disease in adults. The anti-Thy1.1-induced nephritis model has frequently been used to test potential therapeutic candidates for the treatment of this disease.

**Figure 7.** All-trans-RA limits the number of PCNA-positive glomerular cells in rats with anti-Thy1.1-induced glomerulonephritis (pretreatment protocol). Data are mean ± SEM (n = 9/group). See also Figure 4, C and D.

**Figure 8.** Reduction of glomerular platelet-derived growth factor-B staining in anti-Thy1.1-treated nephritic rats by isotretinoin or all-trans-RA (posttreatment protocol). Data are mean ± SEM (n = 9/group).

**Figure 9.** (A) Reduction of glomerular monocytes/macrophages in anti-Thy1.1-treated nephritic rats treated with isotretinoin (P < 0.005) or all-trans-RA (NS). (B) Percentage of glomeruli with positive staining for fibrin deposition. Fibrin deposition was significantly less in anti-Thy1.1-treated nephritic rats treated with all-trans-RA (P < 0.001).
(22,25,40), although no specific treatment modality has yet been developed for the treatment of human IgA nephritis. Much work needs to be performed before the clinical application of retinoids for the treatment of human renal disease might be warranted. However, the pharmacologic features of retinoids have been well described, specific retinoid receptor agonists and antagonists are available, and clinical experience with retinoid treatment of human diseases has already been accumulated. These pharmacologic tools may facilitate the detailed analysis of retinoid effects in renal disease.

Acknowledgments

We thank Dr. M. Klaus and Dr. P. Hadvary (Hofmann-LaRoche, Basel, Switzerland) for providing us with isotretinoin.

References
