Effects of Retinoids on the TGF-β System and Extracellular Matrix in Experimental Glomerulonephritis

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Abstract. Transforming growth factor–β1 (TGF-β1) overexpression plays a key role in the glomerular accumulation of extracellular matrix proteins in renal disease. Retinoids have previously been shown to significantly limit glomerular damage in rat experimental glomerulonephritis. Therefore, the effects of all-trans retinoic acid and isotretinoin on the components of the TGF-β system and extracellular matrix proteins in anti-Thy1.1-nephritis (Thy-GN) were investigated. Vehicle-injected control rats were compared with rats treated with daily subcutaneous injections of 10 mg/kg body wt all-trans retinoic acid or 40 mg/kg body wt isotretinoin (n = 9 per group) either with a pretreatment (day −2 through 8) or posttreatment protocol (day +3 through 8), i.e., starting before or after induction of Thy-GN, respectively. Urinary TGF-β1 excretion was 60% lower in all-trans retinoic acid–treated animals with Thy-GN (P < 0.025). The increase of cortical TGF-β1 gene expression in Thy-GN rats was significantly attenuated with all-trans retinoic acid and even more with isotretinoin treatment as compared with untreated animals (P < 0.025). Cortical expression of TGF receptor II, but not receptor I gene expression, was significantly lower in animals treated with all-trans retinoic acid or isotretinoin (P < 0.05). In all-trans retinoic acid–treated animals with Thy-GN, the increase of glomerular TGF-β1 protein (P < 0.008) and TGF-β1 (P < 0.025) and TGF receptor II mRNA (P < 0.015) was significantly less. Immunohistochemistry revealed less glomerular staining for TGF-β1 and TGF receptor II in the presence of all-trans retinoic acid. TGF-β1 immunostaining was not restricted to monocytes and macrophages, as indicated by double-staining. Glomerular staining for collagen IV and collagen III was less in animals treated with isotretinoin (P < 0.02 for both) in contrast to all-trans retinoic acid, whereas fibronectin remained unchanged. It was concluded that the beneficial effects of retinoids on glomerular damage are presumably due to a marked reduction in renal TGF-β1 and TGF receptor II expression.

Transforming growth factor–β (TGF-β) is a prototype of a profibrogenic cytokine (1). TGF-β stimulates transcription of many extracellular matrix genes in renal cells (2,3). TGF-β inhibits extracellular matrix turnover by reducing collagenase production and by stimulation of tissue inhibitor metalloproteinase expression (4). In several models of renal disease, TGF-β has been implicated as a primary mediator of cell growth and accumulation of extracellular matrix, e.g., diabetic nephropathy, experimental glomerulonephritis, or unilateral ureteral obstruction (5,6). Renal TGF-β1 gene expression is markedly elevated in anti-Thy1.1-nephritis or anti-thymocyte serum nephritis (7–9). Matrix expansion was less when TGF-β1 was antagonized by neutralizing antibodies or antisense oligonucleotides (10,11).

The role of retinoids, derivatives of vitamin A, in embryogenesis (including renal development) has been studied in detail (12,13). Little is known, however, about the renal effects of these compounds. Retinoids exert strong antiproliferative and anti-inflammatory effects. They act via retinoid acid and retinoid X receptor subtypes, which belong to the steroid supergene family (14). These receptors influence gene expression either via retinoid responsive cis-acting elements or via modulation of transcription factors such as AP-1, NFκB, or GATA (15–19).

We had demonstrated that retinoids attenuate glomerular damage in anti-Thy1.1-nephritis (Thy-GN) (20), as indicated by preservation of renal function, less albuminuria, and lower capillary occlusion score. It has been suggested that in this model TGF-β is crucial for matrix expansion and fibrosis. We, therefore, decided to examine the effects of retinoids on the renal TGF-β system and the expression of collagen III, IV, and fibronectin in Thy-GN in the rat. We compared non-nephritic control rats with Thy-GN animals that were treated with all-trans retinoic acid, isotretinoin, or vehicle.

Materials and Methods

Animal Studies

Thy-GN was induced by a single injection of OX-7, a monoclonal antibody against the Thy1.1-antigen (European Collection of Animal...
Cell Cultures, Salisbury, UK). Male pair-fed Wistar rats (Charles River, Sulzfeld, Germany), weighing 180 to 200 g, were used for all studies. In a first experiment (the pretreatment protocol), two groups of 18 animals each were treated with daily subcutaneous injections of 10 mg/kg body wt all-trans retinoic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in arachis oil and 5% dimethylsulfoxide or arachis oil and dimethylsulfoxide as vehicle control, respectively. Two days later, animals received by injection into the tail vein either 1 mg/kg body wt OX-7 (n = 9) or phosphate-buffered saline (PBS) as control (n = 9), respectively. The experiment was terminated 8 d after injection of the antibody.

In a second experiment (the posttreatment protocol), animals were first given OX-7 or PBS and then, 2 d later, 10 mg/kg body wt all-trans retinoic acid or vehicle. This experiment included two further experimental groups that were treated with daily subcutaneous injections of 40 mg/kg body wt isotretinoin (13-cis retinoic acid; Hoffmann LaRoche, Basel, Switzerland) dissolved in arachis oil and 5% dimethylsulfoxide. Each group was composed of nine animals. The experiment was terminated 8 d after injection of the antibody.

BP was determined on day 8 by tail-cuff plethysmography under light ether anesthesia. For determination of TGF-β1 in urine, rats were placed in metabolic cages and urine was collected for 24 h. The experiment was terminated by injection of 5 mg/kg body wt Xylazine intramuscularly (BayerVital, Leverkusen, Germany) and 100 mg/kg body wt Ketamin 10% intramuscularly (WDT, Garbsen, Germany). Kidneys were perfused with normal saline at 37°C that contained 0.5 g/L procaine hydrochloride at a pressure of 110 mm Hg. Glomeruli were isolated by a fractionated sieving technique. The yield and the purity of isolated glomeruli at each time point were comparable between groups (purity >90%).

**RNA Isolation and Reverse Transcription**

The Trizol (Life Technologies, Paisley, UK) method was used for RNA isolation, according to the manufacturer’s recommendations. Samples were checked for degradation of total RNA on 1% agarose gel. RNA concentrations were determined by spectrophotometric measurements at wavelengths of 260/280 nm. Reverse transcription was performed as described elsewhere (21). For each sample, reverse transcription was carried out three times and the resulting cDNA was pooled.

**Quantitative PCR Assay**

Quantification of specific mRNA was performed essentially as described by Wagner et al. (21). For each gene, a DNA deletion mutant was cloned. These had the same sequences as the endogenous genes, with identical primer binding sites but a deletion of maximally 20% that resulted in a shorter amplification product. Reverse-transcribed RNA (0.25 μg) was used for amplification in the presence of defined concentrations of DNA deletion mutants as internal standards. The concentration of standard DNA was selected to allow comparable degrees of amplification of endogenous and mutant genes. Primers were used for TGF-β1 5’-CACCATCATTAGACATGAACC-3’ (sense primer) and 5’-TCTATGGTGACAACGTCC-3’ (antisense), for TGF receptor II 5’-CTACAGGCGCAAGCTGAAGC-3’ (sense) and 5’-AGGCATGAGATGACATCGC-3’ (antisense), and for TGF receptor I (subtype R4) 5’-ATGGAATCAGCTGTAGGCGC-3’ (sense) and 5’-TCAACGGATGATGACAGG-3’ (antisense).

The PCR reaction mix contained 0.25 mM dNTP, 2.5 mM MgCl2, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 80 nM levels of sense and antisense primers, and 1 U of Taq DNA Polymerase (Life Technologies). The thermal profile that was used consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min carried out 30 times for TGF-β1, 28 times for TGF receptor II, and 32 times for TGF receptor I (subtype R4). In all experiments, possible contamination with genomic DNA was excluded by PCR amplification in the absence of reverse transcriptase. Amplification products were separated by agarose gel electrophoresis and then digitized by use of a gel documentation system (Intas, Göttingen, Germany) and NIH Image (National Institutes of Health, Bethesda, MD). The ratio between the optical density of the endogenous cDNA and the optical density of the mutant DNA was determined. Each sample was measured in three individual PCR assays for each gene.

**Enzyme-Linked Immunosorbent Assay for TGF-β1**

For enzyme-linked immunosorbent assay (ELISA), glomeruli were isolated and centrifuged in PBS. The pellet was resuspended in 50 μl of Laemmli buffer 1 (33% 0.5 mM Tris-HCl [pH 6.8], 66% SDS 10%). Protein was isolated by three cycles of freeze and thaw. The supernatant was recovered after centrifugation. The protein concentration was determined according to the method of Bradford (22). TGF-β1 in glomeruli and urine was measured after acid activation by use of a commercially available inhibitory ELISA kit (Immundiagnostik, Bensheim, Germany), according to the manufacturer’s instructions. Each sample was measured in quadruplicate.

**Immunohistochemistry**

Saline-perfused 4-μm slices of renal tissue were fixed in 10% buffered formaline (TGF-β1, TGF receptor II, and Ki-M2R) or methyl Carnoy’s solution (fibronectin and collagen IV and III) and were processed by a direct or indirect immunoperoxidase technique. Primary antibodies included an affinity-purified IgG fraction of a polyclonal rabbit anti-rat fibronectin (Chemicon, Temecula, CA), a biotinylated IgG fraction of a polyclonal goat anti-mouse type IV and type III collagen (Southern Biotech, Birmingham, AL), an IgG fraction of a polyclonal rabbit anti-rat TGF-β1 and TGF receptor II (Santa Cruz Biotechnology, Santa Cruz, CA), and a monoclonal mouse anti-rat pan-macrophage antibody Ki-M2R (DPC Bierrmann, Bad Nauheim, Germany).

Negative controls consisted of substitution of the primary antibody with equivalent concentrations of an irrelevant murine monoclonal antibody or normal rabbit IgG. For each kidney, >30 cross sections of cortical glomeruli with a diameter of at least 100 μm were evaluated in a blinded fashion by use of a 121-point grid (Leitz, Wetzlar, Germany). Points on stained glomerular areas and all points on glomeruli were counted. The ratio in percent was used to determine the degree of staining.

For double-staining of TGF-β1 and Ki-M2R (macrophages), we used the Zymed NBA Kit (Zymed, San Francisco, CA) for TGF-β1 and the DAKO APAAP KIT system (DAKO, Hamburg, Germany) for Ki-M2R, according to the manufacturer’s recommendations.

**Statistical Analyses**

All values are expressed as mean ± SEM. Statistical significance (defined as P < 0.05) was evaluated by use of the nonparametric Mann-Whitney U test.

**Results**

**Effects of Retinoids on Systolic BP**

**Pretreatment Protocol.** Systolic BP was significantly elevated in vehicle-treated rats with Thy-GN on day 8 after induction of disease, compared with controls (control/vehicle,
90 ± 3 mmHg; control/all-trans retinoic acid, 91 ± 4 mmHg; Thy-GN/vehicle, 112 ± 5 mmHg, P < 0.005 versus control/vehicle; Thy-GN/all-trans retinoic acid, 88 ± 3 mmHg, P < 0.001 versus Thy-GN/vehicle). Pretreatment with all-trans retinoic acid had no effect on systolic BP in non-nephritic control rats but completely abrogated the BP increase in Thy-GN rats. BP levels were similar in the posttreatment protocol (data not shown).

**Retinoids Reduce Urinary TGF-β1 Excretion**

**Pretreatment Protocol.** Figure 1 shows a 20-fold increase in urinary TGF-β1 protein in vehicle-treated rats with Thy-GN 8 d after injection of OX-7, compared with controls (P < 0.0004). In Thy-GN rats treated with all-trans retinoic acid, urinary TGF-β1 protein increased only 8-fold (P < 0.025 versus Thy-GN + vehicle). In non-nephritic control rats, all-trans retinoic acid had no effect on urinary TGF-β1 excretion.

**Less Cortical Expression of the TGF-β1 Gene in the Presence of Retinoids**

**Pretreatment Protocol.** Figure 2A shows that treatment with all-trans retinoic acid before injection of OX-7 antibody had no effect on cortical TGF-β1 mRNA expression in non-nephritic animals. Expression of the TGF-β1 gene was markedly higher in the renal cortex of vehicle-treated Thy-GN rats. In Thy-GN animals pretreated with all-trans retinoic acid, TGF-β1 gene expression was significantly less (P < 0.025).

**Post-treatment Protocol.** Cortical TGF-β1 gene expression was also determined in animals that were treated with all-trans retinoic acid or isotretinoin after induction of Thy-GN (Figure 2B). Similar to the pretreatment study, cortical TGF-β1 gene expression was low in Thy-GN rats with all-trans retinoic acid treatment. In comparison, TGF-β1 mRNA was significantly less in isotretinoin than in all-trans retinoic acid–treated Thy-GN rats (P < 0.003). Neither all-trans retinoic acid nor isotretinoin had an effect on cortical TGF-β1 mRNA in non-nephritic control rats.

**Effects of All-Trans Retinoic Acid and Isotretinoin on Cortical Expression of the TGF Receptor Genes**

**Pretreatment Protocol.** In vehicle-treated Thy-GN rats, cortical expression of TGF receptor II was significantly higher (Figure 3A) than in non-nephritic control rats (P < 0.002) but was low after pretreatment with all-trans retinoic acid (P < 0.004). No significant difference was observed between all-trans retinoic acid–treated and vehicle-treated non-nephritic control rats (Figure 3A). In contrast to TGF receptor II, no significant difference in cortical expression of TGF receptor I was observed between vehicle-treated and all-trans retinoic acid–treated Thy-GN rat kidneys.
acid–treated Thy-GN rats. There was also no difference between vehicle-treated control and Thy-GN rats (Figure 3C).

**Posttreatment Protocol.** Cortical TGF receptor II and I gene expression was also determined in animals that were treated with all-trans retinoic acid or isotretinoin after induction of Thy-GN (Figure 3, B and D). The results for both receptors were similar to those obtained in the pretreatment study. The effects of all-trans retinoic acid and isotretinoin on cortical TGF receptor II and I gene expression in Thy-GN rats were not significantly different. Neither all-trans retinoic acid nor isotretinoin had an effect on cortical TGF receptor II and I mRNA in non-nephritic control rats.

**Glomerular TGF-β1 and TGF Receptor II Gene Expression in Thy-GN Rats Treated with All-Trans Retinoic Acid**

**Pretreatment Protocol.** As in the cortex, TGF-β1 gene expression was markedly higher in glomeruli of Thy-GN rats. Expression of this gene was significantly less in animals that were treated with all-trans retinoic acid ($P < 0.025$; Figure 4A). In contrast to TGF-β1 mRNA expression in whole-cortex preparations, gene expression was significantly lower in isolated glomeruli of all-trans retinoic acid–treated non-nephritic control rats when compared with vehicle-treated rats ($P < 0.025$; Figure 4A). Similarly, Figure 4B shows lower TGF

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**Figure 3.** Expression of the TGF receptors in the renal cortex. (A and B) Cortical TGF receptor II expression is less in Ra-treated or Iso-treated Thy-GN rats than in vehicle-treated nephritic rats in the pretreatment (A) and posttreatment (B) protocols. (C and D) No effect of Ra or Iso on cortical TGF receptor I (subtype R4) expression was found in rats with Thy-GN in the pretreatment (C) and posttreatment (D) studies.
receptor II gene expression in glomeruli of all-trans retinoic acid–treated Thy-GN rats than in vehicle-treated Thy-GN animals ($P_{H11021} < 0.015$). Glomerular TGF receptor II gene expression in non-nephritic rats with all-trans retinoic acid treatment was lower than that in vehicle-treated control rats ($P_{H11021} < 0.002$).

**Figure 4.** Ra limits glomerular TGF-β1 and TGF receptor II expression in Thy-GN. (A) Glomerular TGF-β1 gene expression is enhanced in vehicle-treated Thy-GN rats, compared with controls. Glomerular TGF-β1 mRNA is significantly less in the presence of Ra. Glomerular TGF-β1 mRNA is significantly less in Ra-treated control rats versus vehicle-treated control rats. (B) In vehicle-treated Thy-GN rats, glomerular TGF receptor II expression is markedly higher than in control groups but is significantly less when rats were treated with Ra. Glomerular TGF receptor II mRNA is significantly less in Ra-treated control rats versus vehicle-treated control rats.

**Figure 5.** Ra lowers glomerular TGF-β1 protein content. Glomerular TGF-β1 protein is markedly higher in vehicle-treated Thy-GN rats. Ra treatment normalizes glomerular TGF-β1 concentration. No effect of Ra is observed in control rats.

No Increase in Glomerular TGF-β1 Protein in Thy-GN Rats Treated with All-Trans Retinoic Acid

**Pretreatment Protocol.** Figure 5 shows lower glomerular TGF-β1 protein in glomeruli of Thy-GN rats pretreated with all-trans retinoic acid, compared with vehicle-treated Thy-GN rats ($P < 0.008$). TGF-β1 protein content was not significantly different from that of non-nephritic controls. All-trans retinoic acid did not alter glomerular TGF-β1 protein in non-nephritic control rats (Figure 5).

Glomerular Staining for TGF-β1 and TGF Receptor II and Double-Staining for TGF-β1 and Ki-M2R

Figure 6 shows representative examples of glomerular staining for TGF-β1 (Figure 6A through C) and TGF receptor II (Figure 6D through F) in rats with Thy-GN treated with vehicle (Figure 6B and E) or all-trans retinoic acid (Figure 6C, C and F). Figure 6A, A and D shows glomerular staining for TGF-β1 and TGF receptor II in non-nephritic control rats. No significant expression of TGF-β1 protein was found in non-nephritic vehicle-treated glomeruli (Figure 6A). TGF receptor II immunostaining was found in tubular cells but was almost absent in non-nephritic glomeruli (Figure 6D). Staining was not influenced in presence of all-trans retinoic acid in non-nephritic kidneys (data not shown).

In Thy-GN rats, prominent staining for TGF-β1 (Figure 6B) and TGF receptor II (Figure 6E) was noted within the Thy-GN glomeruli, whereas staining was much less in all-trans retinoic acid–treated Thy-GN glomeruli (Figure 6C and F).

Staining of serial sections for TGF-β1 and monocytes/macrophages (Ki-M2R; Figure 6G) as well as double-staining procedures (Figure 6H) showed that TGF-β1 expression in all-trans retinoic acid–treated Thy-GN glomeruli was not exclusively related to monocytes/macrophages but was also found in glomerular areas where no monocyte/macrophage accumulation was evident.

Retinoids Modify the Renal Expression of Extracellular Matrix Proteins

**Posttreatment Protocol.** Figure 7 depicts representative examples of glomerular staining for collagen IV in rats with Thy-GN treated with vehicle (Figure 7B), isotretinoin (Figure...
or all-trans retinoic acid (Figure 7D). Figure 7A shows glomerular staining for collagen IV in control rats. In Thy-GN rats, glomerular staining for collagen IV was markedly higher than in non-nephritic control rats but was significantly less in isotretinoin-treated Thy-GN rats ($P < 0.02$). There was no difference in glomerular collagen IV staining between vehicle-treated and all-trans retinoic acid–treated Thy-GN rats (Figure 8A).

Figure 8B shows no glomerular expression of collagen III in control rats. Collagen III was markedly increased in vehicle-treated Thy-GN rats. Glomerular expression of collagen III was significantly less in presence of isotretinoin than in the vehicle-treated group ($P < 0.02$). In contrast, all-trans retinoic acid had no effect on collagen III expression or even tended to increase it without reaching statistical significance.

Figure 8C shows the result of the quantitative analysis of expression of fibronectin protein. No increase of basal fibronectin expression was seen in control rats in the different treatment groups. Only a slight increase of fibronectin was found in vehicle-treated Thy-GN rats. Isotretinoin had no effect on fibronectin content and was not different from controls. In all-trans retinoic acid–treated Thy-GN rats, there was a significant increase of fibronectin compared with both the isotretinoin-treated and vehicle-treated groups ($P < 0.008$ for both).

**Discussion**

The data document that retinoic acids lower gene and protein expression of the components of the TGF-$\beta$ system in Thy-GN rats and modulate the expression of extracellular matrix proteins in the renal cortex.

The relationship between retinoids and TGF-$\beta$ is complex. Much information has been provided by *in vitro* studies, but the results on the effects of retinoids on the expression of TGF-$\beta$1 have been conflicting; depending on cell type, culture conditions, and type of retinoid used, both increases and decreases have been reported (23,24). Jakowlew *et al.* (25) suggest that the effect of retinoids depends on the basal levels of
TGF-β expression; TGF-β was lowered in cells with high basal expression and increased in cells with low natural abundance of TGF-β. The effects of retinoids on cellular differentiation partly depend on the presence of TGF-β (24). In Hep 2G hepatocarcinoma cells stimulated by 12-O-tetradecanoyl phorbol-13-acetate in vitro, retinoids down-regulate TGF-β via AP-1 binding sites on the TGF-β1 promoter (17).

In vivo data on the effects of retinoids are scarce, although some information is available concerning wound healing and inflammation (26,27). Alteration of body retinoid status resulted in increased TGF-β content in some organs and a decrease in others (28,29).

Many data support a role of TGF-β1 in accumulation of extracellular matrix in Thy-GN (10,11,30,31). A causative role of TGF-β in this model is suggested by studies of Isaka et al. (31), who used a gene transfer system to introduce TGF-β expression vectors into the glomeruli. This maneuver aggravated renal damage. Conversely, blockade of local TGF-β1 synthesis by antisense oligonucleotides or neutralizing antibodies abrogated matrix expansion (10,11). Isaka et al. (30) reported that a TGF receptor II IgG-Fc fusion protein inhibited glomerular damage in this model.

We examined the effects of retinoids on the renal TGF-β system and extracellular matrix proteins because we had previously demonstrated that retinoids strongly reduce glomerular damage in this model (20); retinoids clearly reduce TGF-β1 gene and protein expression on different levels. Urinary TGF-β1 protein is markedly reduced in nephritic rats after exposure to retinoids. Because, however, the pattern of urinary TGF-β1 excretion closely follows the degree of albuminuria, reduction in urinary TGF-β1 may reflect increased tubular reabsorption of TGF-β1 in Thy-GN rats treated with retinoids (20). In whole-cortex preparations and in isolated glomeruli, both TGF-β1 gene expression and TGF-β1 protein were lower in retinoid-treated Thy-GN animals. Immunohistochemistry for TGF-β1 revealed positive staining in mesangial cells and glomerular epithelial cells and segmentally in highly damaged glomerular capillaries. Simultaneously, the glomerular and cortical expression of TGF receptor II was reduced in retinoic acid–treated Thy-GN rats. We cannot decide whether the reduction in TGF receptor II expression is due to the lower number of glomerular cells in all-trans retinoic acid–treated nephritic rats or whether expression per cell is reduced. The latter is probable, given that TGF receptor I mRNA is not altered. Immunohistochemistry also indicated that TGF receptor II staining was less expressed on a per-cell basis. Glomer-

Figure 7. Representative example of immunohistochemical staining for collagen IV in glomeruli of Thy-GN rats. Staining for collagen IV in the glomeruli of non-nephritic rats is shown in vehicle-treated control rats (A) and in Thy-GN rats treated either with vehicle (B), Iso (C), or Ra (D).
ular TGF receptor II immunostaining was found in mesangial cells, glomerular epithelial cells, and podocytes. Gene expression and immunostaining of TGF receptor II (and also of TGF-β1) is enhanced in tubuli of Thy-GN rats. This increase in Thy-GN rats may not reflect a tubular damage in Thy-GN but rather the activation of tubular cells by high protein load, which was also described by others (32,33).

In contrast to the extensive studies on the interaction of retinoids and TGF-β1, little is known about the interaction of retinoids with TGF receptors. Mercier et al. (34) have described down-regulation of TGF receptor II by retinoids. The TGF receptor promoter contains two AP-1 binding sites. Retinoids inhibit AP-1–dependent pathways (35). This suggests, but does not prove, that TGF receptor II expression is reduced by retinoids via an AP-1–dependent mechanism.

The interaction of TGF receptors I and II has not yet been fully understood. There is only one TGF receptor II, but there are different TGF receptor I subtypes, of which involvement with TGF-β signaling has only been demonstrated for subtype R4 (36,37). TGF receptor II binds TGF-β1 and acts as a constitutively active kinase. The complex of TGF receptor II and TGF-β1 is thought to recruit and phosphorylate TGF receptor I, which then initiates the downstream cascade (38,39).

The simultaneous decrease of TGF-β1 and its receptor suggests very effective lowering of TGF-β1 action after treatment with retinoids. This observation supports the notion that the sensitivity of glomerular cells to TGF-β1 is reduced after treatment with retinoids. The effects of retinoids on TGF-β1 were comparable in the pretreatment and the posttreatment protocols, which indicates that the time point of initiation of retinoid treatment does not influence its effect on TGF-β1 expression. Interestingly, however, comparison of the effects of isotretinoin and all-trans retinoic acid shows that isotretinoin reduced the level of TGF-β1 expression significantly more than all-trans retinoic acid. Because we had selected high doses of both compounds, according to the recommendations by Dr. Klaus (Hoffmann-LaRoche), these findings support the idea that retinoids differ in their potency to modulate expression of this cytokine.

The mechanism by which retinoids inhibit TGF-β1 expression cannot be determined from these studies. One has to consider the possibility that lower renal expression of TGF-β1 is only the consequence of reduced glomerular damage in this model. Macrophages and monocytes are well-known sources of TGF-β1, i.e., in acute puromycin aminonucleoside nephrothesis (40). Our data indicate fewer glomerular ED-1 (+) cells in isotretinoin-treated rats (20). However, double-staining for TGF-β1 and monocytes/macrophages (Ki2MR) demonstrated that TGF-β1 is also expressed in glomerular cells other than monocytes/macrophages. This indicates that reduction in glomerular monocyte/macrophage count is not the only explanation for the reduced TGF-β1 staining (Figure 6, G and H). All-trans retinoic acid completely abrogated the BP increase in this model. Because shear stress is known to induce TGF-β1 expression (41), the beneficial effects of retinoids might be, at least in part, the consequence of normalized BP. The finding of lower glomerular TGF-β1 gene expression in non-nephritic rats treated with all-trans retinoic acid suggests, however, a direct effect of retinoids on glomerular TGF-β1 independent from BP and shear stress.

Retinoids exert strong anti–AP-1 activity. TGF-β1 expression depends at least in part on this transcription factor. The TGF-β1 promoter contains three AP-1 consensus sites that mediate retinoid-dependent suppression of TPA-stimulated TGF-β1 gene activation (17). Haxsen et al. (42) demonstrated that retinoids lower basal TGF-β1 gene expression and inhibit angiotensin II induction of TGF-β1 expression in vascular smooth muscle cells. Morishita et al. (43) demonstrated that angiotensin II induction of TGF-β1 gene expression depends

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**Figure 8.** Glomerular staining score of extracellular matrix proteins. Significantly lower collagen IV (A) and collagen III (B) staining was found in glomeruli of rats with Thy-GN after posttreatment with Iso. The staining for fibronectin was higher in Thy-GN rats than in controls (C). Treatment with Ra had no discernible effect on staining for collagen IV (A) and collagen III (B) but led to high staining for fibronectin in Thy-GN rats (C).
on AP-1 activation. These findings suggest that retinoids may also have a direct effect on TGF-β1 via these pathways.

Retinoids also influence the expression of glomerular matrix proteins (44–47). Retinoids are capable of either increasing or decreasing expression of extracellular matrix proteins. Some matrix proteins, e.g., laminin, contain retinoic acid–responsive elements on their promoter (46,47), whereas others do not, i.e., fibronectin and collagen III. Scita et al. (44) demonstrated that retinoic acid reduced fibronectin levels in NIH-3T3 cells. Taub et al. (45) demonstrated a significant reduction of laminin, collagen IV, and heparan sulfate in canine kidney cells after exposure to retinoic acid.

Renal induction of collagen III and IV and fibronectin in Thy-GN rats in our experiments is in accordance with other data obtained in this model (48,49). Parallel to the decrease in renal TGF-β1 and TGF receptor II in Thy-GN rats, glomerular collagen III and IV were decreased after isotretinoin treatment, whereas fibronectin was not influenced. This indicates that isotretinoin has an overall antifibrotic effect in this model. This is in accordance with studies of Shigematsu and Tajima (50), who found that the antifibrotic action of isotretinoin was stronger than that of retinoic acid. In contrast, although all-trans retinoic acid reduced glomerular TGF-β1, it did not reduce collagen III or IV. It evoked a slight but significant increase in fibronectin. From these data, it cannot be safely concluded that all-trans retinoic acid and isotretinoin have differential effects on extracellular matrix proteins. Differences in retinoid metabolism or retinoid receptor activation or shifts in dose-response curves may explain such different effects. Studies on vascular smooth muscle cells, however, have indicated that, at the same concentration, all-trans retinoic acid and isotretinoin, indeed, have different effects on TGF-β1 and procollagen IV expression (preliminary unpublished observations), as has already been proposed by Shigematsu and Tajima (50).

In future experiments, it will be necessary to evaluate whether the various retinoids, i.e., specific retinoid receptor agonists, have different effects and to establish their dose-response relationships. In conclusion, the above data indicate that the beneficial effects of retinoids on glomerular damage in Thy-GN are in part due to reduction of renal TGF-β1 gene expression. Our findings clearly warrant further studies to assess the potential therapeutic effects of retinoids in renal disease, to delineate the mechanisms of retinoid action, and to identify retinoid receptor–specific pathways in the kidney.

References


