Kidney International, Vol. 47 (1995), pp. 62–69

Modulation of experimental mesangial proliferative nephritis by interferon-γ

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Modulation of experimental mesangial proliferative nephritis by interferon-γ. The observation that interferon-γ (IFN-γ) inhibits cell proliferation and collagen synthesis of a variety of cell types in culture has suggested that IFN-γ may be useful in the treatment of fibroproliferative diseases. We administered recombinant IFN-γ subcutaneously (10^5 U/kg/day for 3 days) to rats, beginning one day after the induction of mesangial proliferative nephritis with anti-Thy 1 antibody. IFN-γ reduced glomerular (primarily mesangial) cell proliferation by 44% at days 2 and 4 compared to vehicle injected control rats with anti-Thy 1 nephritis (that is, proliferating cells that excluded the macrophage marker, ED-1, $P < 0.001$). Despite the inhibition of mesangial cell proliferation, IFN-γ did not reduce the overall extracellular matrix deposition (by silver stain) or deposition of type IV collagen or laminin (by immunostaining) at 4 or 7 days, and glomerular type IV collagen and laminin mRNA levels were increased (1.4 and 1.7-fold) at 4 days relative to controls. The inability of IFN-γ treatment to reduce mesangial matrix expansion may relate to the fact that IFN-γ treated rats had a twofold increase in glomerular macrophages (that is, ED-1 positive cells, $P < 0.001$ at 2 and 4 days) with an increase in oxidant producing cells (day 2, $P < 0.05$) and a 1.6-fold increase in glomerular TGF-β mRNA expression (4 days). This suggests that the effect of IFN-γ to inhibit mesangial cell proliferation in glomerulonephritis may be offset by the ability of IFN-γ to increase glomerular macrophages and TGF-β expression. These data also show that IFN-γ can partly dissociate the mesangial proliferative response from the extracellular matrix expansion in glomerulonephritis.

Mesangial cell proliferation can be documented in numerous experimental and human glomerular diseases [1–6]. The mesangial cell proliferation in these diseases is associated with a phenotypic change in which the mesangial cells express proteins characteristic of vascular smooth muscle cells (that is, α-smooth muscle actin) and fibroblasts (that is, type I interstitial collagen) [2, 5–8]. This has suggested to us that the activated mesangial cells in these lesions may be acquiring characteristics of "myofibroblasts" [9].

Although it is possible that some mesangial cell proliferation and phenotypic changes are of benefit to the host in the normal reparative response to glomerular injury, there is evidence that excessive cell proliferation may have a pathogenic role in the development of glomerular sclerosis. First, mesangial cell proliferation precedes and is tightly linked to the development of extracellular matrix expansion and/or sclerosis in experimental models of nephritis [2–4, 8, 10]. Mesangial cell proliferation may also persist in experimental [2, 4] and human [6] glomerular diseases associated with advanced glomerulosclerosis. Finally, measures that reduce cell proliferation in experimental disease models, such as treatment with heparin [11], low protein diet [10], or neutralizing antibody to platelet-derived growth factor (PDGF) [12], also reduce the extracellular matrix expansion or sclerosis.

We were therefore interested in examining the effects of agents that might inhibit mesangial cell proliferation in a model of mesangial proliferative nephritis. Several substances have been reported to inhibit mesangial cell proliferation *in vitro*, including heparin, heparan sulfate proteoglycan, nitric oxide (endothelium derived relaxing factor), atrial natriuretic factor, and interferon-γ (IFN-γ) [13–17, and reviewed in 18]. Treatment with IFN-γ is especially appealing as this cytokine is not only anti-proliferative for a variety of cell types [16–22], but also inhibits other properties observed in myofibroblasts, such as α-smooth muscle actin expression (in smooth muscle cells and fibroblasts) [19, 23, 24] and collagen production (in fibroblasts) [25–28]. Indeed, IFN-γ inhibits cell proliferation, α-smooth muscle actin expression, and collagen production in cultured hepatic lipocytes [23], a cell that is remarkably analogous to the mesangial cell in the way it responds in fibrosing hepatic diseases [29]. We now report the effect of IFN-γ treatment in a rat model of experimental mesangial proliferative nephritis.

Methods

Experimental protocol

Mesangial proliferative nephritis was induced in 150 g male Wistar rats (Simonsen Laboratories, Gilroy, CA, USA) by intravenous injection with antibody to the Thy 1 antigen on the mesangial cell membrane (0.50 ml anti-Thy 1 plasma/100 g body wt) as previously described [7]. Recombinant rat IFN-γ (10^5 U/kg in 0.2 ml normal saline, gift of Peter van der Meide, TNO Primate Center, Rijswijk, The Netherlands) or normal saline alone was then injected subcutaneously at 20, 44, and 68 hours after disease induction. IFN-γ or vehicle injected rats underwent kidney biopsies at days 2 ($N = 6$ per group), 4 ($N = 10$ per group) and day 7

Received for publication May 19, 1994
and in revised form July 27, 1994
Accepted for publication July 28, 1994

($N = 4$ per group). No rat was biopsied more than two times. At days 4 and 7 glomeruli were isolated and RNA extracted for Northern analysis. Serum (day 4) and urine (day 3 to 4) were also collected for blood urea nitrogen and urine protein measurements. Finally, normal rats were injected with similar doses of IFN-γ ($N = 2$) or vehicle ($N = 4$) and biopsied at days 4 and 7 using identical handling and housing procedures as that performed on rats with anti-Thy 1 disease.

Immunostaining

Methyl Carnoy's fixed tissue was processed and 4 μm sections were stained with the periodic acid/Schiff reagent and counterstained with hematoxylin. Additional sections from each biopsy were immunostained by an avidin/biotin indirect immunoperoxidase method [7] with the following primary antibodies: 19A2 (Coulter, Hialeah, FL, USA), a murine monoclonal IgM to the proliferating cell nuclear antigen (PCNA; [30]); ED-1 (Bioproducts for Science, Indianapolis, IN, USA), a murine monoclonal IgG to monocyte-macrophages [31]; RP-3 (gift of F. Sendo, Yamagata, Japan), a monoclonal IgM to rat neutrophils [32]; OX-22 (Accurate Chemical Corporation, Westbury, NY, USA), a monoclonal IgG to the high molecular weight form of the common leukocyte antigen expressed on B-lymphocytes and some T-lymphocytes; αsm-1, a monoclonal IgG to the NH_2-terminal synthetic decapeptide of α-smooth muscle actin [33]; polyclonal rabbit anti-rat laminin (Chemicon, Temecula, CA, USA); polyclonal guinea pig anti-rat type I collagen (gift of L. Iruela-Arispe; [34]); and polyclonal rabbit anti-mouse type IV collagen (Collaborative Research, Bedford, MA, USA) [8]. Tissue was also double immunostained for both proliferating cells (PCNA) and macrophages (with ED-1) by an indirect immunogold procedure as described [1, 35]. Negative controls consisted of replacing the primary antibody with either an irrelevant murine monoclonal or rabbit polyclonal antibody.

Kidney tissue from the day 2 biopsies was also snap-frozen in liquid nitrogen and 6 μm sections stained for hydrogen peroxide produced by activated neutrophils and monocytes. Hydrogen peroxide-generating cells were identified by reaction of the hydrogen peroxide with diaminobenzidine in the presence of myeloperoxidase [36], the latter which is present in neutrophils and monocytes.

Quantitation of proliferating (PCNA positive) and oxidant positive cells and of the various leukocyte populations within glomeruli was performed by enumerating the number of positively stained cells in 20 or more sequentially examined glomerular cross sections per biopsy in blinded manner as previously described [1, 7, 35]. Glomeruli were also graded semiquantitatively (scale 0 to 4+) for the degree of mesangial staining of types I and IV collagen, laminin, and α-smooth muscle actin in blinded fashion as previously described [8]. For α-smooth muscle actin, scoring was as follows: 0, no α actin present; 1+, up to 25% of glomerular tuft positive; 2+, 25 to 50% of glomerular tuft positive; 3+, 50 to 75% positive; 4+, 75 to 100% of tuft positive. For the extracellular matrix components, scoring was as follows: 0, absent mesangial matrix staining (observed only with type I collagen); 1+, diffuse and weak mesangial staining with 1 to 25% of glomerular tuft showing local increased staining; 2+, 25 to 50% of glomerular tuft with focal strong staining; 3+, 50 to 75% of glomerular tuft with strong staining; 4+, > 75% of glomerular tuft with increased staining. Scores primarily reflect change in distribution rather than intensity of mesangial staining, and representative glomeruli with different scores of collagen IV staining are shown in Figure 1. For each biopsy, a mean score was calculated and was then used to generate a mean ± SD for each group.

Northern analysis

Glomeruli were isolated from three to four rats from each group at days 4 and 7, pooled, and the total RNA extracted [7] with RNAzol B (Cinna/Biotecx Laboratories, Friendswood, Texas, USA) according to the manufacturer's instructions and further purified by LiCl precipitation as previously described [2]. For Northern analysis the RNA was denatured and 15 μg/lane were electrophoresed through a 1% formaldehyde/agarose gel and transferred to a nylon filter (Hybond N, Amersham, Arlington Heights, IL, USA) by capillary blotting as described [35]. The following cDNA probes were [^{32}P]dCTP-labeled by random primer extension: for PDGF B-chain, linearized plasmid p3-4a, a clone containing almost the full length of rat PDGF B-chain cDNA (gift of C. Giachelli, Seattle, WA, USA); for PDGF β receptor, a 5.1 kb *Eco*R1 fragment of mouse PDGF β receptor cDNA (gift of D. Bowen-Pope, Seattle, WA, USA) [37]; for TGF-β1, a 985 bp *Hind*III/*Xba*I cDNA fragment from the plasmid rat TGF-β1 (gift of A. Roberts, National Institutes of Health) [38]; for laminin B2, a 1.7 kb *Eco*RI/*Xba*I fragment of mouse laminin B2 cDNA from plasmid p1298 [39]; for type I collagen, an *Eco*R1 fragment of human α1(I) collagen from cDNA clone HF 677 (gift of M. Chu) [40]; for type IV collagen, a 1.8 kb *Eco*RI/*Hind*III fragment of mouse α1 (IV) collagen cDNA from plasmid pPE123 (gift of M. Kurkinen) [41]; and for 28S ribosomal RNA, a 280 bp *Eco*RI cDNA fragment from bovine 28S RNA (gift of L. Iruela-Arispe, Univ. of WA, Seattle, WA, USA) [7]. Two to five Northern blots were performed with each probe. Some membranes were stripped and re-probed. Densitometry readings from autoradiograms were normalized to 28S ribosomal RNA, and the ratio of IFN-γ to control was reported as "relative change" [7].

Other measurements

Blood urea nitrogen was measured in serum samples by autoanalyzer. Urine protein was measured on 24-hour urine specimens collected in metabolic cages using a sulfosalicylic acid method with whole serum standards (Lab Trol, Dade Diagnostics, Aquado, Puerto Rico) as described previously [2].

Statistical analysis

All values shown are mean ± SD. Statistical significance (defined as $P < 0.05$) was evaluated using the Student's t-test.

Results

The injection of anti-Thy 1 antibody resulted in the typical course of mesangial cell loss and matrix disruption (maximal in the first 24 hours) followed by a phase of mesangial cell proliferation and matrix expansion [1, 7, 8, 11].

Effect of IFN-γ on mesangial cell proliferation, leukocyte accumulation, and mesangial phenotype change

Cell proliferation was assessed by immunostaining tissue sections for PCNA, a nuclear accessory protein to DNA polymerase delta which is expressed from late G1 to M phase of the cell cycle

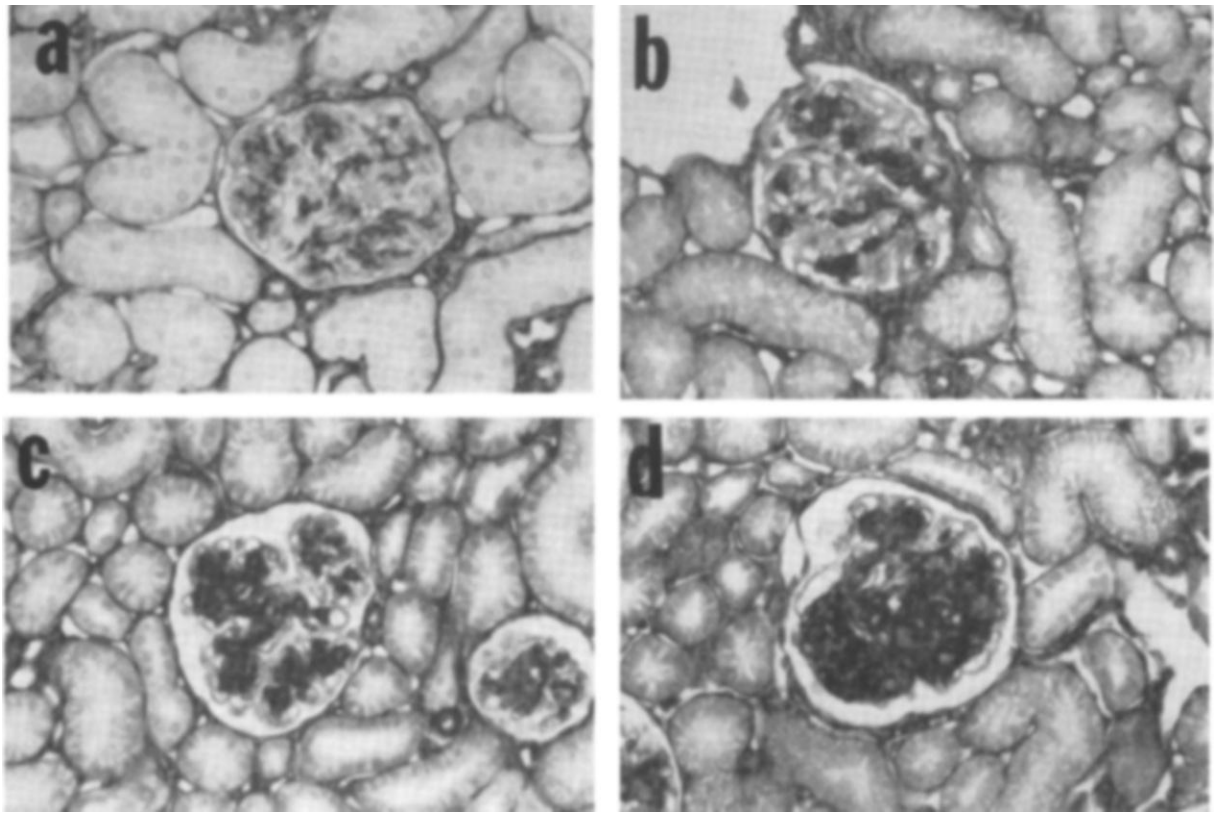

Fig. 1. *Shown are representative glomeruli with different scores for type IV collagen.* The scoring system is detailed in the Methods Section. Glomeruli are shown with scores of 1 (**A**), 2 (**B**), 3 (**C**), and 4 (**D**). Scoring is based primarily on distribution rather than intensity (400×, immunoperoxidase).

[42, 43], and which has been previously shown by us to correlate with cell proliferation in rat kidney tissue as measured by uptake of ^{3}H-thymidine [44]. Treatment with IFN-γ had only a minor effect on total cell proliferation, as noted by a mild reduction of PCNA positive cells at day 4 (Table 1).

In contrast, IFN-γ treatment dramatically increased the glomerular macrophage accumulation by nearly twofold at days 2 and 4 (Table 1). This effect was no longer present four days after the last IFN-γ injection (that is, day 7 after induction of anti-Thy 1 disease) (Table 1).

In previous studies we have documented that 85% of the proliferating cells in this model are glomerular (primarily mesangial) cells whereas 15% are proliferating macrophages [1, 7, 8, 11]. In order to determine if this were also true in rats treated with IFN-γ, tissue was double immunostained for both PCNA and monocyte-macrophages (that is, ED-1). In comparison to vehicle-injected rats with anti-Thy 1 nephritis, IFN-γ treated rats with anti-Thy 1 nephritis had a two- to threefold increase in proliferating macrophages, which represented 35 to 42% of the total proliferating cells at days 2 and 4 (Table 1). In contrast, IFN-γ treated rats with anti-Thy 1 disease had a 44% reduction in proliferating cells that excluded the macrophage marker at days 2 and 4 (Table 1). This strongly suggests that IFN-γ reduced mesangial cell proliferation.

A characteristic of mesangial cells in both experimental and human mesangial proliferative glomerulonephritis is their upregulated (in human) or *de novo* (in rat) expression of α-smooth muscle actin [6, 7]. Despite reducing mesangial cell proliferation, IFN-γ treatment did not significantly reduce mesangial α-smooth muscle actin immunostaining at any time (such as, 2.1 ± 0.6 vs. 2.2 ± 0.6 in IFN-γ vs. vehicle-injected rats with anti-Thy 1 nephritis, day 4, scale 0 to 4+, P = NS).

The increase in glomerular monocyte/macrophages observed in IFN-treated rats was also parallelled by an increase in cells

Table 1. Effect of IFN-γ on cell proliferation and macrophage accumulation in glomeruli of rats with mesangial proliferative nephritis

	Day 2		Day 4	
	Vehicle	IFN-γ	Vehicle	IFN-γ
Total PCNA + cells	16.5 ± 2.2	14.4 ± 1.5	17.7 ± 2.0	13.4 ± 2.5[b]
PCNA+, ED-1− cells	13.7 ± 1.9	8.3 ± 0.9[c]	16.0 ± 2.0	8.8 ± 2.1[c]
PCNA+, ED-1+ cells	2.8 ± 0.9	6.1 ± 0.8[c]	1.7 ± 0.7	4.6 ± 1.3[c]
Total ED-1+ cells	5.8 ± 0.9	10.4 ± 1.9[c]	3.9 ± 2.1	7.5 ± 2.1[a]
N	5	6	6	6
	Day 7			
	Vehicle	IFN-γ		
Total PCNA+	4.4 ± 1	3.8 ± 1		
Total ED-1+	5.2 ± 1	5.5 ± 2		
N	4	4		

Values shown represent the number of positive cells per glomerular cross section. For comparison, normal glomeruli have 0.9 ± 0.2 PCNA-positive cells and 2.7 ± 0.3 ED-1 positive cells per glomerular cross section [33].

[a] $P < 0.02$; [b] $P < 0.001$; [c] $P < 0.001$ comparing vehicle-injected to IFN-γ-injected rats with anti-Thy 1 disease at similar time points

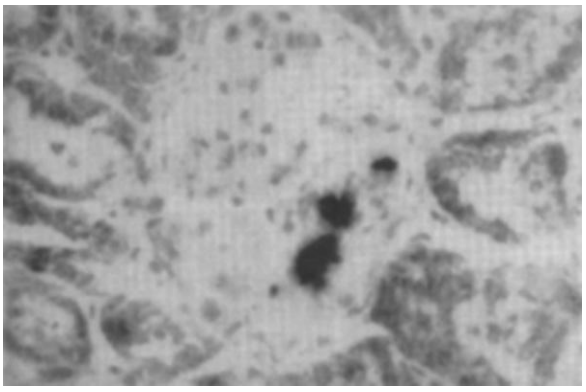

Fig. 2. *Light microscopy showing the presence of hydrogen peroxide secreting cells in the glomerulus of a rat with anti-Thy 1 nephritis that has been treated with IFN-γ (400×).*

secreting hydrogen peroxide. In normal rats, hydrogen peroxide-positive cells are rare (0.4 ± 0.2 cells/glomerular cross section, $N = 4$). However, in rats with anti-Thy 1 glomerulonephritis, an increase in hydrogen peroxide-positive cells was observed, which was even greater in IFN-γ treated rats (0.56 ± 0.32 vs. 1.02 ± 0.22 cells/glomerular cross section in controls vs. IFN-γ treated rats with anti-Thy 1 disease, day 2, $N = 5$ per group, $P < 0.05$; Fig. 2). There was also an increase in the percent of glomeruli in IFN-γ treated rats that had two or more hydrogen peroxide-positive cells (14 ± 9% vs. 29 ± 11% in controls vs. IFN-γ treated rats with anti-Thy 1 disease, $P < 0.05$).

IFN-γ treatment also increased the number of OX-22 positive leukocytes (that is, primarily lymphocytes) in glomeruli at day 4 relative to disease controls (1.0 ± 0.4 vs. 0.7 ± 0.2 OX-22+ cells/glomerular cross section, respectively, $P < 0.05$). However, no difference was noted at day 7 (data not shown). Neutrophils (as detected by the monoclonal antibody, RP-3) were scarce in both vehicle and IFN-γ treated rats with anti-Thy 1 disease (averaging 0.1 cell/glomerular cross section).

Effect of IFN-γ on glomerular expression of mRNA for PDGF B chain and TGF-β

Northern analysis was performed for several growth factors and matrix components on total glomerular RNA isolated from IFN-γ and vehicle-treated rats with anti-Thy 1 nephritis (Fig. 3). PDGF B-chain and TGF-β mRNA, which are up-regulated in anti-Thy 1 nephritis [35, 45], were further increased 1.6- to 1.8-fold by IFN-γ treatment at day 4 (Fig. 3). In contrast, PDGF β receptor mRNA, which is also up-regulated in anti-Thy 1 nephritis [35], was not further enhanced by IFN-γ treatment (Fig. 3). The increased expression of PDGF and TGF-β mRNA in IFN-γ treated rats with anti-Thy 1 nephritis was no longer observed at day 7 (that is, 4 days after the IFN-γ injections had been stopped; data not shown).

Effect of IFN-γ treatment on extracellular matrix expansion in mesangial proliferative nephritis

Both PAS and silver-stained tissue sections showed comparable degrees of increased mesangial matrix expansion in IFN-γ or vehicle-injected rats with anti-Thy 1 nephritis at days 4 and 7. Many glomeruli in both IFN-γ and vehicle treated rats with

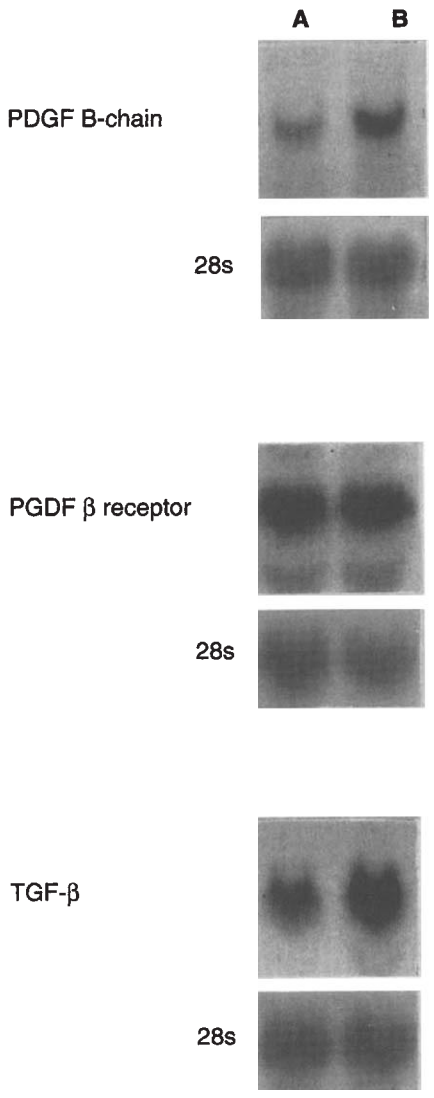

Fig. 3. *Effect of IFN-γ on growth factor mRNA in anti-Thy 1 nephritis at day 4.* Shown are Northern blots of PDGF B chain, PDGF β receptor, and TGF-β mRNA and 28S ribosomal RNA in rats with anti-Thy 1 nephritis that have been treated with vehicle **(A)** or IFN-γ **(B)**. For each blot, densitometry scores were normalized for 28S ribosomal RNA and the relative change in densitometry between IFN-γ treated rats and disease controls calculated. The mean densitometry score was 1.8 for PDGF B chain mRNA, 1.1 for PDGF β receptor mRNA, and 1.6 for TGF-β mRNA.

anti-Thy 1 nephritis showed loss of normal glomerular architecture in areas of mesangial expansion (Fig. 4). There were also no differences between groups when tissue was immunostained for type I collagen, type IV collagen, or laminin (Table 2). In contrast, type IV collagen and laminin B2 mRNA were increased by 1.4- and 1.7-fold, respectively, in glomerular RNA from rats with nephritis that had received IFN-γ when compared to vehicle-injected controls (Fig. 5).

Effect of IFN-γ treatment on renal function and proteinuria in mesangial proliferative nephritis

Blood urea nitrogen concentrations were marginally higher in IFN-γ treated rats with nephritis, although this was clinically

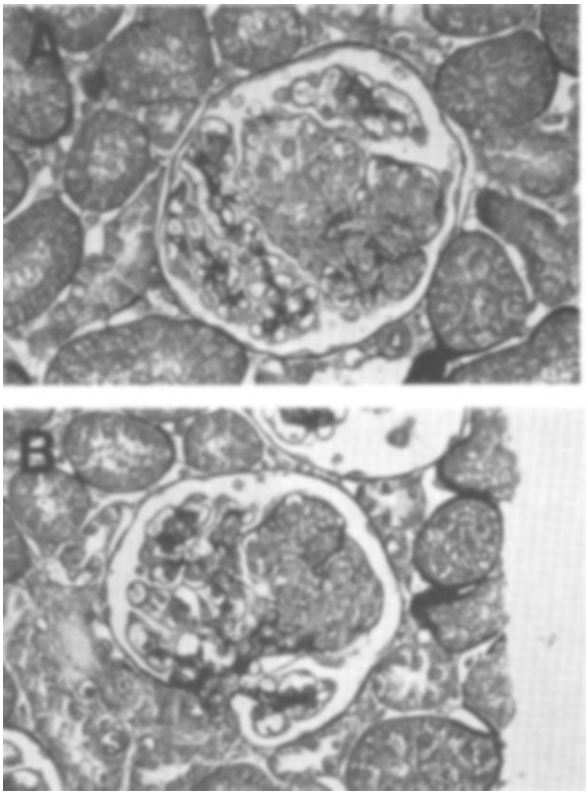

Fig. 4. *Light microscopy of glomeruli from rats with mesangial proliferative nephritis at day 7 that either received vehicle or IFN-γ.* An increase in glomerular cellularity with distortion of glomerular architecture is present in both vehicle **(A)** and IFN-γ treated rats **(B)** with mesangial proliferative nephritis (Jones methenamine silver stain, 400×).

Table 2. Effect of IFN-γ on mesangial matrix expansion of types I and IV collagen and laminin in mesangial proliferative nephritis induced with anti-Thy 1 antibody

	Day 4		Day 7	
	IFN-γ	Vehicle	IFN-γ	Vehicle
Type IV collagen	2.8 ± 0.3	2.9 ± 0.3	2.2 ± 0.3	2.5 ± 0.4
Type I collagen	1.4 ± 0.3	1.6 ± 0.7	1.4 ± 0.2	1.3 ± 0.6
Laminin	2.7 ± 0.2	2.7 ± 0.4	2.8 ± 0.3	2.6 ± 0.4

Values shown represent a mean ± SD of a semi-quantitative grade (0 to 4+) for mesangial matrix expansion as determined by immunostaining with specific antibodies [8]. Comparable values in normal rats (of same age and weight) are, for type IV collagen, 2.2 ± 0.1; for type I collagen, 0.1 ± 0.03; and for laminin 2.0 ± 0.1.

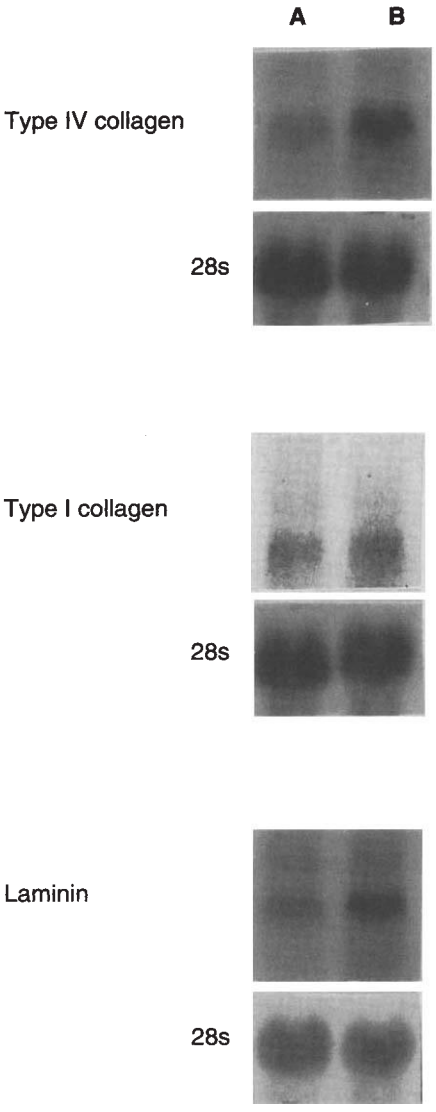

Fig. 5. *Effect of IFN-γ on extracellular matrix mRNA in anti-Thy 1 nephritis at day 4.* Shown are Northern blots for types α1 (IV) and α1(I) collagen and laminin B2 mRNA and 28S ribosomal RNA in rats with anti-Thy 1 nephritis that have been treated with vehicle **(A)** or IFN-γ **(B)**. For each blot, densitometry scores were normalized for 28S ribosomal RNA and the relative change in densitometry between IFN-γ treated rats and disease controls calculated. The mean densitometry score was 1.4 for type IV collagen [α1 (IV)] mRNA, 1.2 for type I collagen [α1 (I)] mRNA, and 1.7 for laminin B2 mRNA.

insignificant (23 ± 2 vs. 20 ± 1 mg/dl in IFN-γ vs. vehicle-injected rats with nephritis, day 4, $P < 0.02$). Proteinuria was no different in IFN-γ treated versus control rats with nephritis (33 ± 20 vs. 25 ± 9 mg/24 hrs in IFN-γ vs. vehicle, days 3 to 4, P = NS).

Effect of IFN-γ on normal rats

Limited studies were also performed in which normal rats either received IFN-γ or saline vehicle. IFN-γ did not induce a macrophage influx or affect cell proliferation (PCNA). Normal rats treated with IFN-γ also had no difference in type IV collagen and laminin by immunostaining compared to normal rats.

Discussion

IFN-γ is a multipotent cytokine produced by activated T cells and NK cells that has anti-proliferative, immunomodulatory, and anti-viral effects [46]. We administered recombinant rat IFN-γ (10^5 U/kg) to rats with mesangial proliferative nephritis induced by anti-Thy 1 antibody in order to determine if it would have a beneficial effect on the disease by reducing mesangial cell proliferation, phenotype change, and matrix expansion. The IFN-γ injections were started one day after the disease induction so as to eliminate concerns over whether or not the IFN-γ would affect the

initial binding of the anti-Thy 1 antibody (which is maximal at 1 hr [47]) or with the acute complement-mediated mesangiolysis. Initiation of IFN-γ injections after disease induction is also of more relevance to the question of whether or not IFN-γ may be clinically useful in the treatment of glomerular disease.

Treatment with IFN-γ only mildly reduced total glomerular cell proliferation (that is, PCNA-positive cells) at day 4. However, these proliferating cells could be separated by double immunostaining into those cells that were proliferating macrophages (that is, PCNA +, ED-1 +) and proliferating cells that excluded the macrophage marker (that is, PCNA +, ED-1 −) which are likely glomerular (and primarily mesangial) cells. Using this technique, one could demonstrate a significant increase in proliferating macrophages in glomeruli with a concomitant reduction (44%) in other proliferating cells at both days 2 and 4. That this reduction in proliferating cells represented primarily mesangial cells is supported indirectly by the minimal numbers of other leukocytes in these glomeruli, and by our previous studies which have demonstrated that the majority of the PCNA-positive cells in this model express Thy 1 (a marker for mesangial cells) and α-smooth muscle actin (a marker for activated mesangial cells) [1, 7, 8, 11, 35]. However, a contribution by other endogenous glomerular cells (that is, endothelial cells) cannot be excluded.

The mechanism for the anti-proliferative effect of interferon on the mesangial cell in this model may be direct, as it is known that IFN-γ may inhibit the proliferation of a variety of cell types in culture, including mesangial cells, smooth muscle cells, and fibroblasts [16–21]. Indeed, IFN-γ inhibits the proliferative response of rat mesangial cells to PDGF [17, and RJ Johnson et al, unpublished data], which has been shown to be an important mediator of cell proliferation in this model [12, 35]. Studies in human fibroblasts suggest that the inhibitory effects of IFN-γ on PDGF-mediated cell proliferation occur distal to the PDGF receptor [21]. This could potentially explain why IFN-γ inhibited the glomerular cell proliferation in this model despite inducing an increase in PDGF B-chain mRNA expression.

The inhibitory effects of IFN-γ on glomerular cell proliferation may also be indirect. For example, TGF-β mRNA expression was up-regulated in glomeruli of rats with IFN-γ treatment. TGF-β inhibits mesangial cell proliferation under most conditions *in vitro* [48, 49] despite the fact that TGF-β also induces PDGF mRNA expression in these cells [48]. IFN-γ also activates macrophages to produce nitric oxide (endothelium-derived relaxing factor) [50], which is a potent inhibitor of mesangial cell proliferation to a variety of mitogens, including PDGF [reviewed in 51]. Other inhibitory factors may also be induced. For example, we have evidence that SPARC/osteonectin, which can inhibit mesangial cell proliferation in culture, is induced by IFN-γ treatment in this model (unpublished observations).

Despite the inhibition of mesangial cell proliferation, there was no significant reduction of α-smooth muscle actin expression. We had originally reported that α-smooth muscle actin was a marker for proliferating mesangial cells in glomerulonephritis [7]. However, we have subsequently been able to show that the α-smooth muscle actin expression and mesangial cell proliferation can be dissociated; for example, with angiotensin II infusion (in which α-actin is expressed despite minimal mesangial cell proliferation [44]) or with PDGF infusion (in which mesangial cell proliferation occurs without α-smooth muscle actin expression [52]). Whereas we had expected that IFN-γ would reduce both the mesangial cell proliferation and α-actin expression, it is possible that IFN-γ may have inhibited proliferation, but due to expression of other factors, such as TGF-β, that the phenotype was not altered. Recently it has been reported that TGF-β induces α-smooth muscle actin in granulation tissue myofibroblasts and in cultured fibroblasts [53].

The second major finding was that treatment of mesangial proliferative nephritis with IFN-γ resulted in a near doubling of the monocyte/macrophage accumulation in glomeruli. Many of these monocyte-macrophages were expressing the proliferation-associated marker, PCNA, suggesting that they were proliferating in the glomeruli or had arrived in the glomeruli shortly after proliferating in the marrow or lymph nodes. An increase in cells expressing hydrogen peroxide was also observed. These oxidant-producing cells were most likely monocytes. This is based on the observation that only neutrophils and monocytes contain the endogenous peroxidase (that is, myeloperoxidase) necessary for the assay to detect hydrogen peroxide and neutrophils were not present in these lesions. It is also known that IFN-γ significantly increases the cytotoxicity and oxidant production by monocyte/macrophages in response to stimuli [54].

It is possible that the increased localization of activated macrophages in this model counteracted the effects of IFN-γ on reducing the mesangial cell proliferation. Indeed, the original hypothesis, that a reduction in mesangial cell proliferation would translate into a reduction in extracellular matrix, was not observed, as immunostaining for matrix proteins was not different between groups, and matrix mRNA levels were in fact increased in IFN-γ treated animals. We [55] and others [56, 57] have previously shown a tight correlation between macrophage influx, matrix expansion, and glomerular sclerosis in other experimental models of nephritis. If macrophages are responsible for the increase in extracellular matrix, the mechanism could relate to expression of TGF-β by the macrophage itself, or to macrophage stimulation of other (such as, mesangial) cells to express TGF-β. TGF-β stimulates the production of extracellular matrix by a variety of cells, including mesangial cells [49, 58], and has been shown to be a mediator of matrix expansion in the anti-Thy 1 model [59].

This study also provides insights into potential discrepancies between *in vitro* and *in vivo* data in relation to IFN-γ. The findings that IFN-γ inhibits cell proliferation [16–21, 23] and collagen production [25–28] of a variety of cell types in culture suggest that it may be useful in the treatment of fibroproliferative diseases such as interstitial pneumonitis, fibrosing hepatic diseases and glomerulonephritis. However, lupus mice (the NZB/NZW F_1 hybrid) treated with IFN-γ have increased proteinuria, onset of glomerulonephritis, and mortality [60, 61]. Increased glomerular cellularity and sclerosis was also observed with IFN-γ treatment in a murine model of IgA nephropathy [62]. However, the effects of IFN-γ on disease processes may also vary depending on the specific disease process or the tissue involved.

In conclusion, IFN-γ has multiple effects in experimental glomerulonephritis. While it has anti-proliferative effects on glomerular cells, it also increases glomerular macrophage localization and activation. In addition to characterizing the effects of IFN-γ in nephritis, this study provides some interesting insights into glomerular biology. First, it provides evidence that significant numbers of macrophages in glomeruli may express proliferation markers (which is of relevance in all studies in which glomerular

cell proliferation is examined). Second, it demonstrates that cell proliferation and matrix expansion are not necessarily concordant, but can be partially dissociated. And finally, it provides further, albeit indirect, evidence that matrix expansion may be linked to macrophages and TGF-β expression.

Acknowledgments

This study was supported in part by U.S. Public Health Service Grants DK-43422 DK-34198, DK-07467, and DK-02142, and by the Swiss National Science Foundation (Grant Nr.31-30796.91)

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Appendix

Abbreviations used in this paper are: IFN-γ, interferon-γ; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β.

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