American Journal of Pathology, Vol. 151, No. 1, July 1997

Role of Interleukin-1 in Mesangial Cell Proliferation and Matrix Deposition in Experimental Mesangioproliferative Nephritis

Gregory H. Tesch, Hui Y. Lan, Robert C. Atkins, and David J. Nikolic-Paterson

From the Department of Nephrology, Monash Medical Centre, Clayton Victoria, Australia

We examined the functional role of interleukin (IL)-1 in mesangial cell proliferation during rat anti-Thy-1 nephritis by blocking its action with IL-1 receptor antagonist (IL-1ra). Anti-Thy-1 nephritis was induced by intravenous injection of 5 mg/kg OX-7 IgG (day 0) into inbred Wistar rats. Groups of animals (n = 9) were implanted with a micro-osmotic pump on day −1, which delivered 25 μg/hour human recombinant IL-1ra or saline continuously until the rats were killed at day 6, the peak of mesangial cell proliferation. Immunostaining showed that IL-1 was expressed by mesangial cells during disease. IL-1ra treatment did not affect the mild, but significant, proteinuria seen after OX-7 injection. Compared with saline treatment, IL-1ra treatment reduced mesangial cell proliferation (↓24%; P < 0.05), glomerular hypercellularity (↓29%; P < 0.05), and glomerular macrophage accumulation (↓20%; P < 0.05). However, IL-1ra treatment had no effect on glomerular IL-1β mRNA expression and caused only a small reduction in the high levels of glomerular expression of platelet-derived growth factor-β protein (↓6%; P < 0.05). IL-1ra caused a modest reduction in the marked upregulation of glomerular transforming growth factor-β1 mRNA expression on day 6 (↓26%; P < 0.05), although urinary excretion of this factor was unaffected. Interestingly, IL-1ra treatment had relatively little effect upon glomerular deposition of laminin, fibronectin, and collagen type IV seen in this acute disease. In conclusion, this study has 1) demonstrated that IL-1 is expressed by mesangial cells in vivo, 2) demonstrated that IL-1 is a mesangial cell growth factor in experimental mesangioproliferative nephritis, and 3) suggests that IL-1 has little or no fibrogenic activity in mesangial matrix deposition. (Am J Pathol 1997, 151:141–150)

Mesangial cell proliferation is a pathological feature of glomerulonephritis, and several animal models have shown that mesangial cell proliferation precedes the development of glomerulosclerosis.[1–3] Interleukin (IL)-1 was one of the first growth factors identified for mesangial cells *in vitro*.[4] Mesangial cells can also synthesize IL-1,[5–7] suggesting that IL-1 can stimulate mesangial cell growth through autocrine and/or paracrine pathways. In addition, IL-1 has been shown to stimulate the production of a number of other mesangial cell growth factors, including platelet-derived growth factor (PDGF), basic fibroblast growth factor (FGF-2), and IL-6.[8–11]

Glomerular IL-1 expression is increased in various animal models of glomerulonephritis,[5,12–14] and blockade of IL-1 action by administration of IL-1 receptor antagonist (IL-1ra) has been shown to reduce glomerular cell proliferation and hypercellularity.[15] Also, studies of human IgA nephritis have demonstrated glomerular IL-1 expression,[16,17] with the number of IL-1$^+$ cells correlating with mesangial hypercellularity.[16] However, the issue of whether IL-1 acts as a mesangial cell growth factor in mesangioproliferative disease has not been addressed, nor is it clear whether mesangial cells can produce IL-1 in disease.

Another possible role for IL-1 in mesangioproliferative disease is in promoting glomerular sclerosis. A number of *in vitro* studies have suggested that IL-1 is a pro-fibrotic factor.[18] For example, IL-1 has been shown to promote proliferation of mesangial cells

Supported by the National Health and Medical Research Council (grant 930827), the Baxter Extramural Program, and a Monash University Scholarship (G. H. Tesch).

Accepted for publication April 18, 1997.

Address reprint requests to Dr. David J. Nikolic-Paterson, Department of Nephrology, Clayton Road, Clayton, Victoria 3168, Australia.

and renal fibroblasts *in vitro*[4,19] and to stimulate synthesis of laminin and collagen in cultured glomerular epithelial cells,[20,21] although it is uncertain whether IL-1 can stimulate fibroblast collagen synthesis.[22,23] Treatment of experimental models of bleomycin-induced pulmonary fibrosis and crescentic glomerulonephritis with the IL-1ra have suppressed tissue fibrosis,[15,24] although these studies did not clarify whether IL-1 was acting to cause tissue damage to which fibrosis was the response or whether IL-1 was acting directly as a fibrogenic growth factor.

We have examined the role of IL-1 in rat anti-Thy-1 disease, a model of mesangioproliferative nephritis, to determine whether 1) mesangial cells express IL-1 *in vivo*, 2) IL-1 is a mesangial cell growth factor *in vivo*, and 3) IL-1 can directly promote glomerular matrix deposition.

Materials and Methods

Rat Anti-Thy-1 Nephritis

Acute anti-Thy-1 nephritis was induced in male Wistar inbred rats (150 to 170 g) by intravenous injection of 5 mg/kg OX-7 IgG. Groups of three animals were sacrificed on days 1, 4, 6, 8, 10, 14, and 21 after OX-7 injection to determine the time course of mesangial proliferation and IL-1β expression. In addition, groups of nine animals given OX-7 injection were treated with a constant infusion of either human recombinant IL-1ra (hrIL-1ra; 25 μg/hour at 0.5 μl/hour) or normal saline (at 0.5 μl/hour) via a microosmotic pump (Alzet 1007D, Alza Corp., Palo Alto, CA) implanted subcutaneously under the back of the neck from 24 hours before OX-7 IgG injection until animals were killed on day 6. The hrIL-1ra was generously supplied by Amgen, Boulder, CO. A group of nine normal rats was also examined.

Proteinuria and Renal Function Assessment

Twenty-four-hour urine collections and blood samples were taken on days −3 (before experiment), 1, 3, and 6. Urinary protein concentration was measured by the benzethonium chloride method.[25] Serum and urine creatinine levels were measured using the Jaffe rate reaction.[26]

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum levels of hrIL-1ra were quantitated on days 1, 3, and 6 in animals treated with hrIL-1ra by a commercial ELISA kit (Quantikine DRA00, R&D Systems, Minneapolis, MN). In addition, urine levels of TGF-β1 were measured in normal rats and experimental animals on day 6 using an ELISA kit (G1230, Promega, Madison, WI). All assays were carried out according to the manufacturer's protocols.

Histochemical Analysis

Tissues were fixed in 4% neutral buffered formalin and embedded in paraffin. Kidney sections (3 μm) were stained with periodic-acid Schiffs reagent and counterstained with Harris's hematoxylin to detect cell nuclei. Glomerular hypercellularity was determined by counting the number of nuclei in 10 hilar glomerular tuft cross sections per animal.

Antibodies

The following monoclonal antibodies were used: OX-7, anti-rat Thy-1.1 CDw90[27]; MCA 1397, anti-rat interleukin-1β (Serotec, Oxford, UK)[28]; OX-1, anti-rat CD45 common leukocyte antigen[29]; ED1, anti-rat monocytes and macrophages, CD68[30]; RECA-1, pan rat endothelial cell marker, (HIS52, Serotec)[31]; 1A4, anti-human α-smooth muscle actin (anti-α-SMA; Sigma Immunochemicals, St. Louis, MO); PC-10, anti-proliferating cell nuclear antigen (anti-PCNA; Dakopatts, Glostrup, Denmark)[32]; and mouse anti-human CD45R, IgG1 (73.5), and mouse anti-human CD45, IgG2a (71.5) were used as isotype controls. Polyclonal antibodies used were rabbit anti-human PDGF-β (ZP-215, Genzyme, Cambridge, MA); rabbit anti-rat laminin IgG (AB1901, Silenus Laboratories, Melbourne, Australia); rabbit anti-rat fibronectin IgG (AB1942, Silenus); and rabbit anti-mouse collagen IV (40025, Collaborative Biomedical Products, Bedford, MA). In addition, to prevent antibody cross-reactivity while staining frozen sections, OX-1 and OX-7 monoclonal antibodies were conjugated with digoxigenin (DIG) according to the manufacturer's instructions (Boehringer Mannheim, Mannheim, Germany). Secondary polyclonal antibodies used were goat anti-mouse IgG conjugated with horseradish peroxidase (HRP), (Dakopatts); rabbit anti-mouse IgG2a-HRP (Zymed Laboratories, San Francisco, CA); sheep anti-rabbit IgG-HRP (Silenus); sheep anti-DIG IgG Fab fragments-HRP (Boehringer Mannheim); HRP-conjugated mouse (and rabbit) anti-HRP IgG complexes (mPAP and rPAP, Dakopatts); goat anti-mouse IgG-alkaline-phosphatase (IgG-AP; Dakopatts); rabbit anti-mouse IgG2a-AP (Zymed); sheep anti-rabbit IgG-AP (Silenus); AP-conjugated mouse (and rabbit)

anti-AP IgG complexes (mAPAAP, Silenus; rAPAAP, Sigma).

Immunohistochemistry

Immunohistochemistry staining was performed on tissues that were fixed by several different methods, including cryostat sections (6 μm) of snap-frozen, ethanol-fixed tissues and tissues fixed in 2% paraformaldehyde-lysine-periodate and paraffin sections (3 or 4 μm) of tissues fixed in 4% neutral buffered formalin or methylcarn solution. For immunostaining of IL-1β, ED1, PCNA, and PDGF-β, tissue sections were first microwave treated for 10 minutes in 0.01 mol/L sodium citrate buffer, pH 6.0, according to a previously described method.[33]

For single antigen immunolabeling, tissue sections were preincubated with 10% fetal calf serum/10% normal sheep serum followed by 5% bovine serum albumin for 20 minutes each and then incubated with the primary antibody in 1% bovine serum albumin overnight at 4°C. DIG-conjugated primary antibodies were incubated in 10% normal rat serum. After labeling, the endogenous peroxidase activity in tissue sections was blocked by incubating in methanol with 0.3% H_2O_2 for 20 minutes. Sections then were labeled with HRP-conjugated second antibody. The non-DIG-labeled sections were incubated for an additional 40 minutes with PAP complexes (mPAP and rPAP) and peroxidase developed with 3,3-diaminobenzidine (Sigma) to produce a brown color.

Those sections being double labeled were next microwave treated to prevent antibody cross-reactivity,[34] preincubated as above, and then incubated with a primary antibody overnight at 4°C. After incubation with the AP-conjugated second antibody, sections were washed and incubated for an additional 40 minutes in APAAP complexes (mAPAAP and rAPAAP) and developed with fast blue BB salt (Ajax Chemicals, Melbourne, Australia) to produce a blue color. Sections were counterstained with periodic acid-Schiffs reagent and mounted in aqueous medium.

Quantitation of Immunohistochemistry

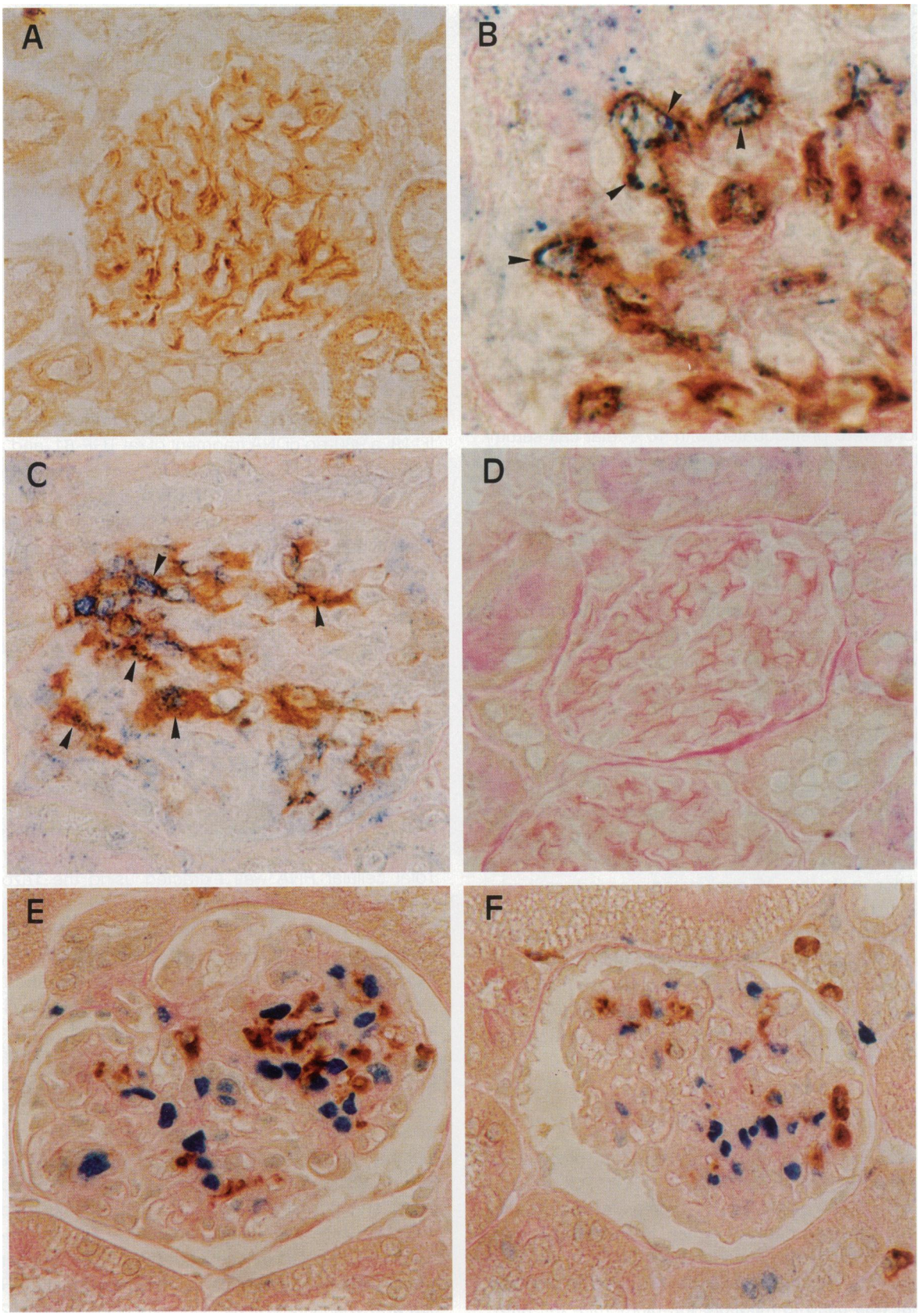
Sections labeled with ED1 and PCNA monoclonal antibodies were scored for ED1$^+$, ED1$^+$PCNA$^+$, and ED1$^-$PCNA$^+$ cells within the glomerular tuft, omitting cells within the capillary lumen, in sections counterstained with periodic acid-Schiffs. Counting was performed under high power (×40) using a graticule that was used to measure the glomerular tuft area. Fifty glomeruli were scored for each animal, and labeled cells were expressed as the mean ± SD per mm^2. In addition, the number of RECA-1$^+$PCNA$^+$, total PDGF-β^+, ED1$^+$PDGF$^+$, and α-SMA$^+$PDGF-β^+ cells were counted in 50 glomerular cross sections per animal in double-immunostained sections. Antibody staining for IL-1β and extracellular matrix proteins (collagen IV, laminin, and fibronectin) was assessed in 20 glomeruli per animal using the following semiquantitative scale: 0 (no labelling), 1 (1 to 10% of glomerular area positive), 2 (10 to 25% positive), 3 (25 to 50% positive), and 4 (>50% positive). All scoring was performed on blinded slides.

Probes

For Northern blotting, a 1.26-kb cDNA fragment of rat IL-1β, a 980-bp cDNA fragment of rat TGF-β1, and a 784-bp fragment of rat 18 S cDNA were prepared by reverse transcription PCR, restriction mapped, and cloned using the pMOSBlue T-vector kit (Amersham International, Little Chalfont, UK; accession number M98829 and Ref. 35). Anti-sense DIG-labeled cRNA probes were prepared using a RNA-labeling kit according to the manufacturer's instructions (Boehringer Mannheim). Incorporation of DIG into the cRNA probes was assessed by dot blot analysis.

Northern Blotting

Glomeruli were isolated from normal and diseased kidneys by sequential sieving to approximately 95% purity, as assessed by phase contrast microscopy. Total cellular RNA was extracted using the Trizol reagent (Gibco BRL Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Northern blotting was performed as previously described.[36] Briefly, RNA samples were denatured, size fractionated on 1.2% agarose gels, and capillary blotted onto Hybond-N membranes (Amersham). Membranes were hybridized overnight at 68 to 73°C with DIG-labeled cRNA probes in 5X SSPE/50% formamide/0.2 mg/ml herring sperm DNA/0.2% SDS/0.1% *N*-lauryl sarcosine. After hybridization, membranes were washed finally in 0.1X SSC/0.1% SDS at 68 to 73°C. Bound probes were detected using sheep anti-DIG antibody (Fab) conjugated with AP, which was developed using CPD-star enhanced chemiluminescence (ECL; Boehringer Mannheim). ECL emissions captured on Kodak XAR film were measured by densitometry using the Cue 2 Image Analyser program (Olympus).


A
B
C
D
E
F

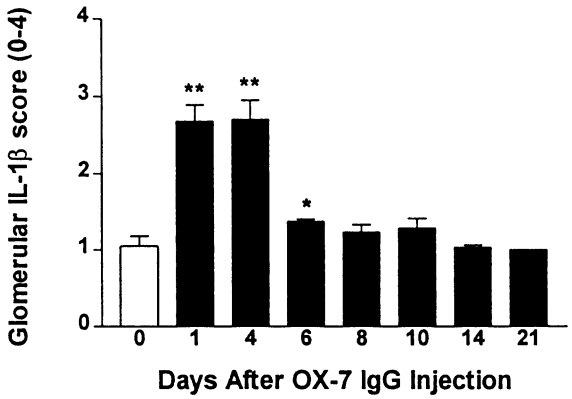


Figure 2. *Semiquantitative assessment of glomerular IL-1β immunostaining in rat anti-Thy-1 nephritis. The glomerular area stained was assessed on a score of 0 to 4 in normal animals (□) and in anti-Thy-1 nephritis (■). Data are shown as mean ± SD.* $^{*}P < 0.05$; $^{**}P < 0.005$ versus *normal.*

Results

Glomerular IL-1β Expression in Anti-Thy-1 Nephritis

Immunohistochemical staining demonstrated constitutive IL-1β expression by glomerular endothelial cells and some mesangial cells in normal rat kidney (Figure 1A). Endothelial expression of IL-1β in normal rat glomeruli was confirmed by double staining with an endothelial cell marker (RECA-1 MAb; Figure 1B). Glomerular IL-1β expression was markedly upregulated at day 1 of rat anti-Thy-1 nephritis (Figure 2), with endothelial staining particularly prominent. The peak of glomerular IL-1β expression occurred at day 4 of disease (Figure 2), which was also the early phase of mesangial cell proliferation and the peak of glomerular macrophage accumulation.[37] As shown in Figure 1C, mesangial cells (OX-7$^+$) were a major source of glomerular IL-1β expression on day 4 of anti-Thy-1 nephritis, whereas few infiltrating leukocytes (OX-1$^+$) were double stained for IL-1β (not shown). Glomerular IL-1β expression declined rapidly after day 4, returning to normal levels by day 8 of disease.

IL-1ra Treatment of Anti-Thy-1 Nephritis

Serum levels of administered hrIL-1ra in anti-Thy-1 nephritis were quantitated by ELISA (Figure 3a).

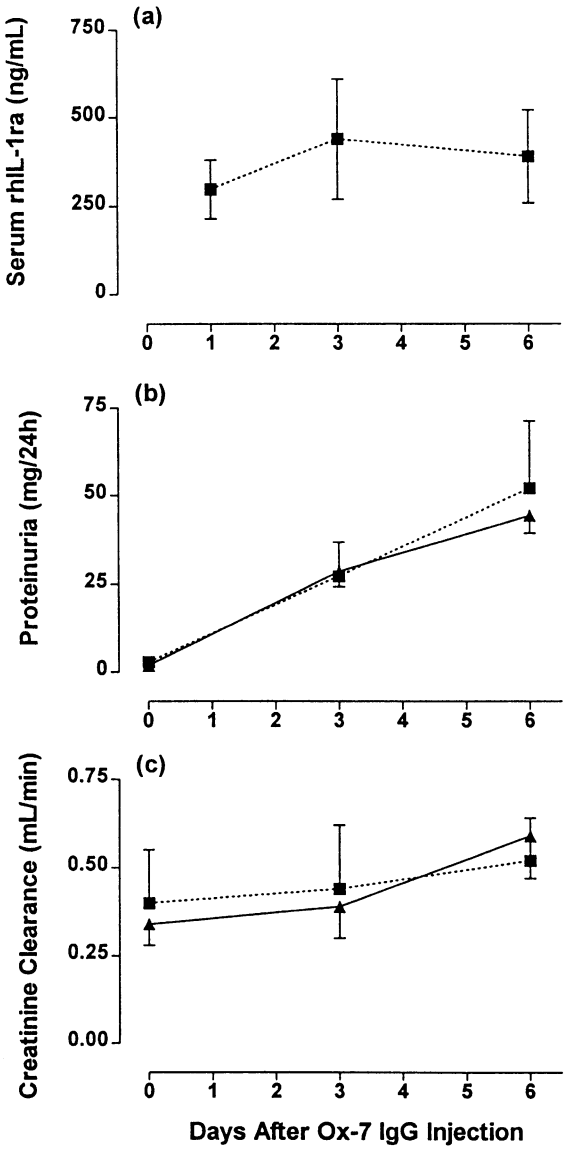


Figure 3. *Effects of IL-1ra treatment on renal function in rat anti-Thy-1 nephritis.* a: *Serum levels of rhIL-1ra in animals treated with constant infusion of rhIL-1ra.* b: *Proteinuria in saline-treated animals (——▲——) and IL-1ra-treated animals (- - -■- - -). Both saline- and IL-1ra-treated animals had significantly elevated proteinuria on days 3 and 6 compared with normals* ($P < 0.0001$), *but there was no difference between saline- and IL-1ra-treated groups.* c: *Creatinine clearance in saline-treated animals (——▲——) and IL-1ra-treated animals (- - -■- - -) was not different from that of normals or between each other.*

High serum concentrations of hrIL-1ra (349 ± 50 ng/ml), similar to those that were shown to suppress experimental crescentic glomerulonephritis,[15] were

Figure 1. *Immunohistochemical staining of glomeruli in rat anti-Thy-1 nephritis.* A: *Antibody staining showing endothelial and some mesangial cell IL-1β expression (brown) in normal rat kidney.* B: *Double immunostaining of normal rat kidney showing co-localization of IL-1β staining (blue) with that of the endothelial cell marker RECA-1 (brown) in glomerular capillaries* (arrowheads). C: *Double immunostaining at day 4 of disease showing IL-1β expression (blue) by OX-7$^+$ mesangial cells (brown); examples of double-stained cells are indicated by* arrowheads. D: *Isotype negative control antibody staining for double-labeling experiments.* E: *Double immunostaining at day 6 of disease showing a segmental area of PCNA$^+$ proliferating cells (blue nuclei) containing numerous ED1$^+$ macrophages (brown) in a saline-treated animal, which is reduced with IL-1ra treatment as shown in* F. *Original magnification, ×400 (*A *and* C *to* F*) and ×1000 (*B*).*

maintained throughout the treatment period. Treatment of anti-Thy-1 disease with IL-1ra had no affect on the mild, but significant, proteinuria seen at days 3 and 6 of disease (Figure 3b). Neither saline- or IL-1ra-treated groups showed any change in creatinine clearance compared with normal rats (Figure 3c).

Mesangial Cell Proliferation

On day 6 of anti-Thy-1 nephritis, the peak of mesangial cell proliferation, saline-treated animals showed moderate glomerular hypercellularity (81.1 ± 3.2 *versus* 60.8 ± 2.1 cells per hilar cross section in normals; $P < 0.01$). Treatment with the IL-1ra reduced glomerular hypercellularity by 29% ($P < 0.05$) compared with saline-treated controls (Figure 4a).

Many glomerular $PCNA^+$ proliferating cells were seen on day 6 of saline-treated anti-Thy-1 nephritis (Figure 1E). Staining of serial sections found that most $PCNA^+$ cells were present in segmental lesions that contained many α-SMA-expressing cells, indicating that these were proliferating mesangial cells (not shown). In addition, immunostaining showed that proliferating $ED1^+$ macrophages and $RECA\text{-}1^+$ endothelial cells accounted for only 6 and 3% of total glomerular $PCNA^+$ cells, respectively. Therefore, mesangial cell proliferation was determined by scoring the number of $ED1^-PCNA^+$ cells per 1000 μm^2 of the glomerular tuft in double-stained tissue sections. Saline-treated animals had 3.8 ± 0.9 $ED1^-PCNA^+$ cells/1000 μm^2 on day 6 of anti-Thy-1 nephritis, which was reduced by 24% ($P < 0.05$) with IL-1ra treatment (Figure 1F and 4b). There was a small, but significant, increase in glomerular tuft area in saline-treated animals (4.8 ± 0.5 *versus* 4.3 ± 0.4 × 1000 μm^2 in saline-treated and normals, respectively; $P < 0.05$), which was not significantly changed with IL-1ra treatment (4.6 ± 0.5 × 1000 μm^2; IL-1ra-treated *versus* saline-treated, P = not significant).

Growth Factor Production and Secretion

Glomerular IL-1β mRNA expression was detectable in normal glomeruli and was markedly up-regulated at day 6 of disease (↑ 370%; $P < 0.005$). There was no significant difference in the level of glomerular IL-1β mRNA expression between saline- and IL-1ra-treated animals (Figure 5, a and b).

Immunohistochemical staining showed weak constitutive expression of PDGF-β protein in glomeruli of normal animals (1.1 ± 0.4 cells per glomerular cross section). There was a marked increase in glomerular

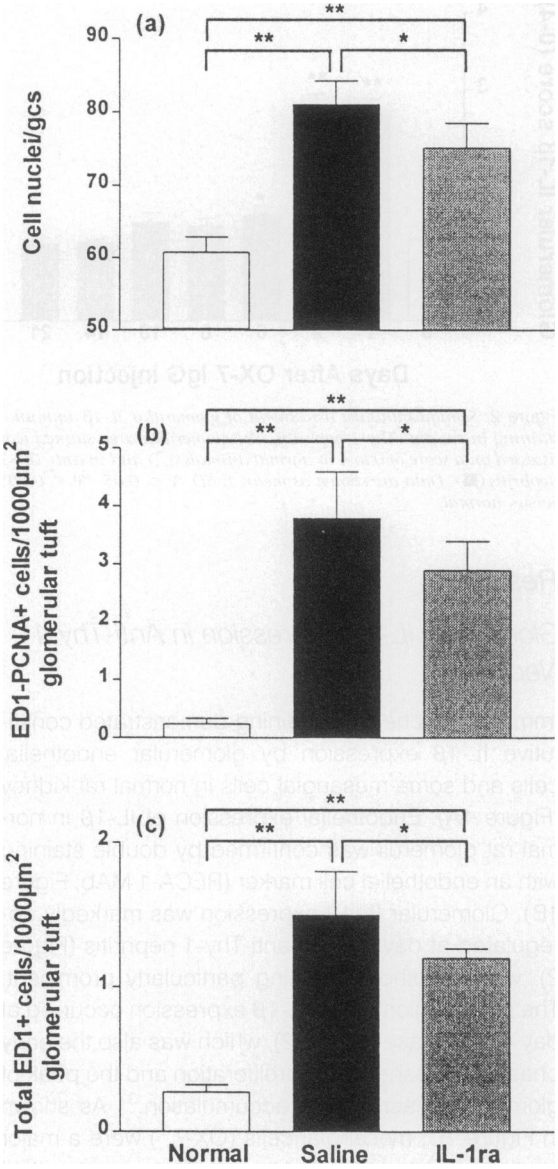


Figure 4. *Effect of IL-1ra treatment on glomerular hypercellularity and mesangial cell proliferation at day 6 in rat anti-Thy-1 nephritis.* **a:** *Glomerular cellularity was determined by counting hematoxylin-stained nuclei from normal* (□), *saline-treated* (■), *and IL-1ra-treated* (**shaded bar**) *animals.* **b:** *Mesangial cell proliferation was determined by counting $ED1^-PCNA^+$ glomerular cells detected by immunostaining in normal* (□), *saline-treated* (■), *and IL-1ra-treated* (**shaded bar**) *animals.* **c:** *Glomerular macrophage accumulation was determined by counting total $ED1^+$ glomerular cells detected by immunostaining in normal* (□), *saline-treated* (■), *and IL-1ra-treated* (**shaded bar**) *animals. Data are shown as mean ± SD.* $^*P < 0.05$; $^{**}P < 0.005$.

PDGF-β staining at day 6 of anti-Thy-1 nephritis (Figure 6), being expressed by mesangial cells, endothelial cells, podocytes, and approximately 10% of macrophages (0.7 ± 0.1 $ED1^+$/PDGF-β^+ cells per glomerular cross section). IL-1ra treatment caused a 6% reduction ($P < 0.05$) in the total number of glomerular PDGF-β^+ cells at day 6 (Figure 6a). Mesan-

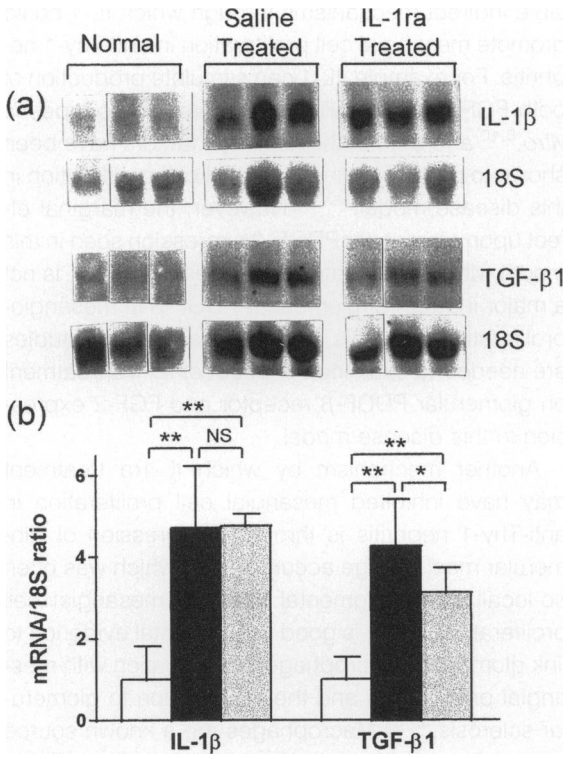


Figure 5. *Glomerular IL-1β and TGF-β1 mRNA expression at day 6 in rat anti-Thy-1 nephritis.* a: *Northern blots showing IL-1β and TGF-β1 mRNA expression in normal, saline-treated, and IL-1ra-treated animals (three examples shown per group). The 18 S rRNA control probe is also shown.* b: *Graph summarizing the densitometry analysis of glomerular IL-1β and TGF-β1 mRNA relative to that of 18 S rRNA. The ratios of IL-1β/18 S and TGF-β/18 S were normalized for normal rats. Data are shown as mean ± SD.* ¶ < 0.05; *¶ < 0.005.

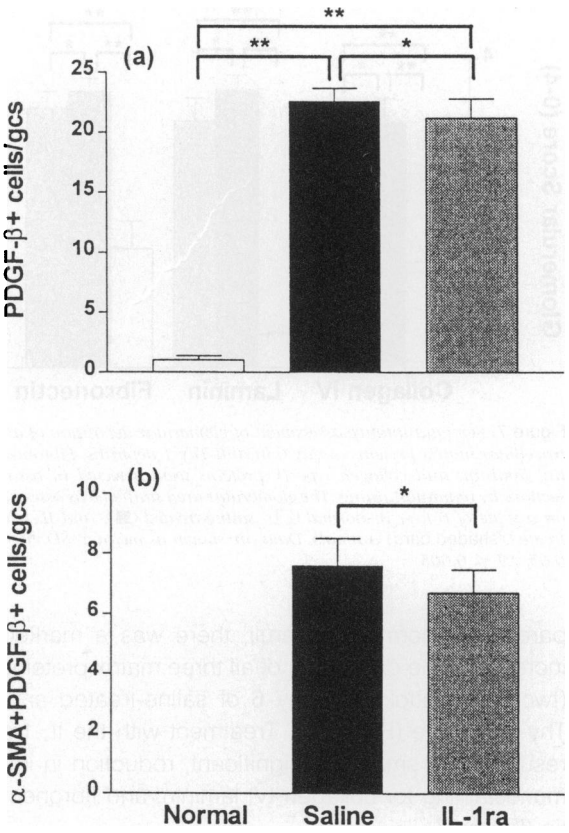


Figure 6. *Glomerular expression of PDGF-β protein at day 6 in rat anti-Thy-1 nephritis.* a: *Glomerular PDGF-β protein expression was detected in tissue sections by immunostaining. The total number of PDGF-β$^+$ cells per glomerular cross section was counted in normal (□), saline-treated (■), and IL-1ra-treated* (shaded bar) *animals.* b: *Tissue sections were double labeled with antibodies to PDGF-β and α-SMA. The number of PDGF-β$^+$ α-SMA$^+$ cells per glomerular cross section were counted in normal (□), saline-treated (■), and IL-1ra-treated* (shaded bar) *animals. Data are shown as mean ± SD.* ¶ < 0.05; *¶ < 0.005.

gial cell expression of PDGF-β was demonstrated by co-localization of PDGF-β and α-SMA (not shown). IL-1ra treatment caused a 12% reduction in the number of mesangial cells expressing PDGF-β compared with saline-treated animals (6.7 ± 0.8 *versus* 7.6 ± 0.9 α-SMA$^+$PDGF-β^+ cells per glomerular cross section in IL-1ra- and saline-treated animals, respectively; $P < 0.05$; Figure 6b).

Glomerular expression of TGF-β1 mRNA was weak in normal animals and was strongly up-regulated (↑ 330%; $P < 0.005$) in saline-treated animals at day 6 of disease (Figure 5). Treatment with IL-1ra caused a modest reduction in glomerular TGF-β1 mRNA (↓ 26%; $P < 0.05$) compared with saline-treated animals. TGF-β1 protein was not detectable in the urine of normal rats (<15 pg/ml) but was easily detected in urine at day 6 in saline-treated rats with anti-Thy-1 disease (887 ± 607 pg/24 hours). IL-1ra treatment had no significant effect upon urinary TGF-β1 excretion (1436 ± 598 pg/24 hours; P = not significant compared with saline-treated).

Glomerular Macrophage Accumulation

There was a significant glomerular ED1$^+$ macrophage infiltrate on day 6 of saline-treated anti-Thy-1 nephritis, which was frequently localized in segmental areas of mesangial cell proliferation (Figures 1E and 4c). Treatment with the IL-1ra inhibited glomerular macrophage accumulation by 20% ($P < 0.05$; Figure 1F). In addition, there was a reduction in the number of ED1$^+$PCNA$^+$ proliferating macrophages in glomeruli of IL-1ra-treated rats, although this was not significant (0.11 ± 0.05 *versus* 0.15 ± 0.07 ED1$^+$PCNA$^+$ cells/1000 μm^2 in IL-1ra- and saline-treated rats, respectively; P = not significant).

Glomerular Extracellular Matrix Deposition

Glomerular deposition of collagen type IV, laminin, and fibronectin was evaluated by semiquantitative scoring of antibody-stained tissue sections. Com-

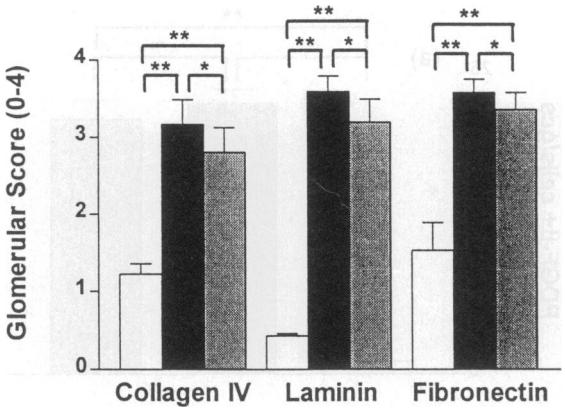


Figure 7. *Semiquantitative assessment of glomerular deposition of extracellular matrix proteins at day 6 in anti-Thy-1 nephritis. Fibronectin, laminin, and collagen type IV proteins were detected in tissue sections by immunostaining. The glomerular area stained was assessed on a scale of 0 to 4 in normal* (□), *saline-treated* (■), *and IL-1ra-treated* (shaded bars) *animals. Data are shown as mean ± SD.* ¶ < 0.05; ** ¶ < 0.005.

pared with normal glomeruli, there was a marked increase in the deposition of all three matrix proteins (two- to eightfold) on day 6 of saline-treated anti-Thy-1 disease (Figure 7). Treatment with the IL-1ra resulted in a small, but significant, reduction in immunostaining for collagen IV, laminin, and fibronectin (Figure 7).

Discussion

This study has demonstrated that IL-1 is expressed by mesangial cells in experimental mesangioproliferative nephritis and that blocking IL-1 activity in this disease model inhibits mesangial cell proliferation. These results have confirmed, and extended, previous studies showing that IL-1 stimulates the proliferation of cultured mesangial cells and that mesangial cells can synthesize IL-1 *in vitro*.[4,6] The two main issues arising from this study are 1) what the mechanisms are by which IL-1 promotes mesangial cell proliferation and 2) whether IL-1 stimulates glomerular extracellular matrix production resulting in sclerosis.

Antibody staining showed that rat glomerular endothelial cells constitutively express IL-1β. There was an increase in glomerular IL-1β mRNA and protein production in anti-Thy-1 nephritis, with antibody staining showing IL-1β expression by mesangial cells, endothelial cells, and some infiltrating macrophages. This is the first direct demonstration of IL-1β production by mesangial cells in disease and indicates that direct IL-1 stimulation of mesangial cell proliferation may operate through autocrine and/or paracrine mechanisms. There are also several possible indirect mechanisms through which IL-1 could promote mesangial cell proliferation in anti-Thy-1 nephritis. For example, IL-1 can stimulate production of both FGF-2 and PDGF-β by different cells types *in vitro*,[8,10] and both of these growth factors have been shown to participate in mesangial cell proliferation in this disease model.[38–41] However, the marginal effect upon glomerular PDGF-β expression seen in this model with IL-1ra treatment suggests that IL-1 is not a major inducer of glomerular PDGF-β in mesangioproliferative nephritis, although additional studies are needed to examine the effect of IL-1ra treatment on glomerular PDGF-β receptor and FGF-2 expression in this disease model.

Another mechanism by which IL-1ra treatment may have inhibited mesangial cell proliferation in anti-Thy-1 nephritis is through suppression of glomerular macrophage accumulation, which was often co-localized in segmental areas of mesangial cell proliferation. There is good experimental evidence to link glomerular macrophage accumulation with mesangial proliferation and the progression to glomerular sclerosis.[42,43] Macrophages are a known source of mesangial cell growth factors such as IL-1, PDGF, and FGF-2,[4,44,45] and therefore, inhibition of macrophage accumulation by IL-1ra treatment may contribute to suppression of mesangial cell proliferation.

The role of IL-1 in fibrosis has been somewhat controversial. *In vitro* studies have given conflicting results in terms of IL-1 stimulating fibroblast collagen synthesis.[22,23] Although administration of IL-1ra has been shown to suppress both pulmonary and renal fibrosis in animal disease models,[15,24] it is unclear whether this effect is due to inhibition of the tissue injury that induces the fibrotic response or whether it is a direct action on the fibrotic process itself. The current study has provided some insight into this issue. The induction of glomerular injury in anti-Thy-1 nephritis, in terms of increased urinary protein excretion, was unaffected by IL-1ra treatment, consistent with the known role of antibody and complement in the induction of this disease.[1,46] This allowed us to examine the role of IL-1 in TGF-β expression and glomerular matrix deposition during the response to glomerular injury. IL-1ra treatment caused a partial reduction in glomerular TGF-β mRNA expression but did not affect the level of activated TGF-β protein in the urine. In addition, IL-1ra treatment had little impact upon glomerular deposition of laminin, fibronectin, or collagen type IV. The small reduction in glomerular matrix deposition is most probably accounted for by the inhibition of mesangial cell proliferation. Therefore, IL-1 appears to have little or no fibrotic activity in this disease model.

In conclusion, this study has demonstrated IL-1 production by mesangial cells *in vivo* and a pathogenic role for IL-1 in mesangial cell proliferation in anti-Thy-1 nephritis. Taken together with the known ability of the IL-1ra to suppress crescentic glomerulonephritis, these results strengthen the case for IL-1ra treatment of human proliferative glomerulonephritis.

Acknowledgments

We thank Rita Foti for the production of riboprobes.

References

1. Floege J, Johnson RJ, Gordon K, Iida H, Pritzl P, Yoshimura A, Campbell C, Alpers CE, Couser WG: Increased synthesis of extracellular matrix in mesangial proliferative nephritis. Kidney Int 1991, 40:477–488
2. Floege J, Burns MW, Alpers CE, Yoshimura A, Pritzl P, Gordon K, Seifert RA, Bowen-Pope DF, Couser WG, Johnson RJ: Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. Kidney Int 1992, 41:297–309
3. Young BA, Johnson RJ, Alpers CE, Eng E, Gordon K, Floege J, Couser WG, Seidel K: Cellular events in the evolution of experimental diabetic nephropathy. Kidney Int 1995, 47:935–944
4. Lovett DH, Ryan JL, Sterzel RB: Stimulation of rat mesangial cell proliferation by macrophage interleukin-1. J Immunol 1983, 131:2830–2836
5. Werber HI, Emancipator SN, Tykocinski ML, Sedor JR: The interleukin-1 gene is expressed by rat glomerular mesangial cells and is augmented in immune complex glomerulonephritis. J Immunol 1987, 138:3207–3212
6. Lovett DH, Szamel M, Ryan JL, Sterzel RB, Gemsa D, Resch K: Interleukin-1 and the glomerular mesangium. I. Purification and characterization of a mesangial cell-derived autogrowth factor. J Immunol 1986, 136:3700–3705
7. Lovett DH, Larsen A: Cell cycle-dependent interleukin-1 gene expression by cultured glomerular mesangial cells. J Clin Invest 1988, 82:115–122
8. Raines EW, Dower SK, Ross R: Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA. Science 1989, 243:393–396
9. Abbott F, Ryan JJ, Ceska M, Matsushima K, Sarraf CE, Rees AJ: Interleukin-1β stimulates human mesangial cells to synthesize and release interleukins-6 and -8. Kidney Int 1991, 40:597–605
10. Francki A, Uciechowski P, Floege J, von der Ohe J, Resch K, Radeke HH: Autocrine growth regulation of human glomerular mesangial cells is primarily mediated by basic fibroblast growth factor. Am J Pathol 1995, 147:1372–1382
11. Sterzel RB, Schulze-Lohoff E, Marx M: Cytokines and mesangial cells. Kidney Int Suppl 1993, 39:S26–S31
12. Boswell JM, Yui MA, Burt DW, Kelley VE: Increased tumor necrosis factor and IL-1β gene expression in the kidneys of mice with lupus nephritis. J Immunol 1988, 141:3050–3054
13. Matsumoto K, Atkins RC: Glomerular cells and macrophages in the progression of experimental focal and segmental glomerulosclerosis. Am J Pathol 1989, 134: 933–945
14. Matsumoto K: Production of interleukin-1 by glomerular macrophages in nephrotoxic serum nephritis. Am J Nephrol 1990, 10:502–506
15. Lan HY, Nikolic-Paterson DJ, Zarama M, Vannice JL, Atkins RC: Suppression of experimental crescentic glomerulonephritis by the interleukin-1 receptor antagonist. Kidney Int 1993, 43:479–485
16. Yoshioka K, Takemura T, Murakami K, Okada M, Yagi K, Miyazato H, Matsushima K, Maki S: *In situ* expression of cytokines in IgA nephritis. Kidney Int 1993, 44:825–833
17. Takemura T, Yoshioka K, Murakami K, Akano N, Okada M, Aya N, Maki S: Cellular localization of inflammatory cytokines in human glomerulonephritis. Virchows Arch 1994, 424:459–464
18. Kovacs EJ: Fibrogenic cytokines: the role of immune mediators in the development of scar tissue. Immunol Today 1991, 12:17–23
19. Lonnemann G, Shapiro L, Engler-Blum G, Muller GA, Koch KM, Dinarello CA: Cytokines in human renal interstitial fibrosis. I. Interleukin-1 is a paracrine growth factor for cultured fibrosis-derived kidney fibroblasts. Kidney Int 1995, 47:837–844
20. Richardson CA, Gordon KL, Couser WG, Bomsztyk K: IL-1β increases laminin B2 chain mRNA levels and activates NF-kappa B in rat glomerular epithelial cells. Am J Physiol 1995, 268:F273–F278
21. Torbohm I, Berger B, Schonermark M, von Kempis J, Rother K, Hansch GM: Modulation of collagen synthesis in human glomerular epithelial cells by interleukin-1. Clin Exp Immunol 1989, 75:427–431
22. Postlethwaite AE, Raghow R, Stricklin GP, Poppleton H, Seyer JM, Kang AH: Modulation of fibroblast functions by interleukin-1: increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin-1 α and β. J Cell Biol 1988, 106:311–318
23. Bathon JM, Hwang JJ, Shin LH, Precht PA, Towns MC, Horton WE Jr: Type VI collagen-specific messenger RNA is expressed constitutively by cultured human synovial fibroblasts and is suppressed by interleukin-1. Arthritis Rheum 1994, 37:1350–1356
24. Piguet PF, Vesin C, Grau GE, Thompson RC: Interleukin-1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. Cytokine 1993, 5:57–61
25. Iwata J, Nishikaze O: New micro-turbidimetric method

for determination of protein in cerebrospinal fluid and urine. Clin Chem 1979, 25:1317–1319
26. Larsen K: Creatinine assay by a reaction-kinetic principle. Clin Chim Acta 1972, 41:209–217
27. Mason DW, Williams AF: The kinetics of antibody binding to membrane antigens in solution and at the cell surface. Biochem J 1980, 187:1–20
28. Schotanus K, Holtkamp GM, Meloen RH, Puijk WC, Berkenbosch F, Tilders FJ: Domains of rat interleukin-1β involved in type I receptor binding. Endocrinology 1995, 136:332–339
29. Sunderland CA, McMaster WR, Williams AF: Purification with monoclonal antibody of a predominant leukocyte-common antigen and glycoprotein from rat thymocytes. Eur J Immunol 1979, 9:155–159
30. Dijkstra CD, Dopp EA, Joling P, Kraal G: The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 1985, 54:589–599
31. Duijvestijn AM, van Goor H, Klatter F, Majoor GD, van Bussel E, van Breda Vriesman PJ: Antibodies defining rat endothelial cells: RECA-1, a pan-endothelial cell-specific monoclonal antibody. Lab Invest 1992, 66: 459–466
32. Waseem NH, Lane DP: Monoclonal antibody analysis of the proliferating cell nuclear antigen (PCNA): structural conservation and the detection of a nucleolar form. J Cell Sci 1990, 96:121–129
33. Tesch GH, Wei M, Ng YY, Atkins RC, Lan HY: Enhancement of immunodetection of cytokines and cytokine receptors in tissue sections using microwave treatment. Cell Vision 1996, 2:435–439
34. Lan HY, Mu W, Nikolic-Paterson DJ, Atkins RC: A novel, simple, reliable, and sensitive method for multiple immunoenzyme staining: use of microwave oven heating to block antibody crossreactivity and retrieve antigens. J Histochem Cytochem 1995, 43:97–102
35. Qian SW, Kondaiah P, Roberts AB, Sporn MB: cDNA cloning by PCR of rat transforming growth factor β-1. Nucleic Acids Res 1990, 18:3059
36. Hattori M, Nikolic-Paterson DJ, Lan HY, Kawaguchi H, Ito K, Atkins RC: Up-regulation of ICAM-1 and VCAM-1 expression during macrophage recruitment in lipid induced glomerular injury in ExHC rats. Nephrology 1995, 1:221–232
37. Nikolic-Paterson DJ, Jun Z, Tesch GH, Lan HY, Foti R, Atkins RC: *De novo* CD44 expression by proliferating mesangial cells in rat anti-Thy-1 nephritis. J Am Soc Nephrol 1996, 7:1006–1014
38. Floege J, Eng E, Lindner V, Alpers CE, Young BA, Reidy MA, Johnson RJ: Rat glomerular mesangial cells synthesize basic fibroblast growth factor: release, up-regulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. J Clin Invest 1992, 90: 2362–2369
39. Floege J, Eng E, Young BA, Alpers CE, Barrett TB, Bowen-Pope DF, Johnson RJ: Infusion of platelet-derived growth factor or basic fibroblast growth factor induces selective glomerular mesangial cell proliferation and matrix accumulation in rats. J Clin Invest 1993, 92:2952–2962
40. Iida H, Seifert R, Alpers CE, Gronwald RG, Phillips PE, Pritzl P, Gordon K, Gown AM, Ross R, Bowen-Pope DF, et al: Platelet-derived growth factor (PDGF) and PDGF receptor are induced in mesangial proliferative nephritis in the rat. Proc Natl Acad Sci USA 1991, 88:6560–6564
41. Johnson RJ, Raines EW, Floege J, Yoshimura A, Pritzl P, Alpers C, Ross R: Inhibition of mesangial cell proliferation and matrix expansion in glomerulonephritis in the rat by antibody to platelet-derived growth factor. J Exp Med 1992, 175:1413–1416
42. Van Goor H, van der Horst ML, Fidler V, Grond J: Glomerular macrophage modulation affects mesangial expansion in the rat after renal ablation. Lab Invest 1992, 66:564–571
43. Diamond JR, Pesek-Diamond I: Sublethal X-irradiation during acute puromycin nephrosis prevents late renal injury: role of macrophages. Am J Physiol 1991, 260: F779–F786
44. Henke C, Marineili W, Jessurun J, Fox J, Harms D, Peterson M, Chiang L, Doran P: Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung injury. Am J Pathol 1993, 143:1189–1199
45. Wangoo A, Taylor IK, Haynes AR, Shaw RJ: Up-regulation of alveolar macrophage platelet-derived growth factor-B (PDGF-B) mRNA by interferon-γ from *Mycobacterium tuberculosis* antigen (PPD)-stimulated lymphocytes. Clin Exp Immunol 1993, 94:43–50
46. Yamamoto T, Wilson CB: Complement dependence of antibody-induced mesangial cell injury in the rat. J Immunol 1987, 138:3758–3765