American Journal of Pathology, Vol. 145, No. 1, July 1994

Glomerular Expression of Interleukin-1 Receptor Antagonist and Interleukin-1β Genes in Antibody-Mediated Glomerulonephritis

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Interleukin-1 (IL-1) is a powerful proinflammatory cytokine whose function is modulated by a natural IL-1 receptor antagonist (IL-1ra). There are few data about kinetics of* in vivo *synthesis of IL-1ra at tissue level, except in response to bacterial endotoxin. The purpose of this study was to examine the kinetics of local expression of IL-1ra gene in relation to IL-1β gene in a model of anti-glomerular basement membrane antibody-mediated glomerulonephritis. Rats were killed in groups of 5 or 6 at 0, 4, 6, 24, 48, and 96 hours after induction of glomerulonephritis. Messenger RNA for IL-1ra and IL-1β was undetectable by Northern blot in normal glomeruli but increased markedly 4 to 6 hours after induction of nephritis. The increase in IL-1ra mRNA was more sustained than that of IL-1β mRNA.* In situ *hybridization showed that IL-1β mRNA increased diffusely within glomeruli, while IL-1ra mRNA was expressed more discretely. Expression of these mRNA in noninflamed tissues, spleens and lungs, was different, particularly increase in IL-1ra mRNA was more substantial than that of IL-1β. These observations suggest that differential expression of IL-1ra and IL-1β might focus inflammation in glomeruli while protecting more distant sites. They also raise the possibility of reducing glomerular injury by therapeutic measures that upregulate glomerular synthesis of IL-1ra while reducing that of IL-1β. (Am J Pathol 1994, 145:126–136)

Interleukin-1 (IL-1) is one of the most powerful substances known to modulate inflammation.[1] It occurs in two forms, IL-1α and IL-1β, both of which have similar affinities for IL-1 receptors and cause inflammation when injected *in vivo*.[2] Substantial amounts of IL-1 are released by monocytes and neutrophils when activated or injured[1,3,4] and smaller amounts by tissue cells, such as fibroblasts and mesangial cells.[5,6] Synthesis of active IL-1 is tightly regulated by transcription[7–10] and translation[11] and through proteolytic cleavage of 31-kd pro-IL-1β, which results in the mature 17.5-kd IL-1β protein.[12] The effects of IL-1 are also limited by a natural interleukin-1 receptor antagonist (IL-1ra), which binds IL-1 receptors but is devoid of agonist activity.[13,14] IL-1 and IL-1ra are often synthesized together, for example by monocytes stimulated with endotoxin,[15] but are differentially regulated. This has been demonstrated by stimulation of monocytes with surface bound IgG, which preferentially induces IL-1ra synthesis.[16] It follows that the balance between IL-1 and IL-1ra concentration will determine the effective IL-1 activity in a given situation, but to date, most of the evidence of *in vivo* co-synthesis of IL-1 and IL-1ra have come from studies in which inflammation has been induced by bacterial products.[17–20] The kinetics of IL-1 and IL-1ra synthesis have not been studied during the evolution of antibody-mediated inflammation.

There is convincing evidence for the involvement of IL-1 in glomerular injury.[21] Steady-state IL-1β mRNA level increased in kidneys of rats with experimental immune complex glomerulonephritis[22] and in lupus-prone mice with nephritis.[23] Glomeruli purified from kidneys with experimentally induced[24] and clinical nephritis[25] released IL-1 when incubated *ex vivo*. Also, we have shown that injection of small doses of human recombinant IL-1β increases injury in the het-

Presented in part at the Annual Meeting of the American Society of Nephrology, Baltimore, MD, in November 1992. F. W. K. Tam is an MRC Training Fellow. S. J. Cashman and Y. Wang are supported by National Kidney Research Fund.

Accepted for publication April 4, 1994.

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erologous phase of nephrotoxic nephritis.[26] Small doses of bacterial lipopolysaccharide (LPS) have the same effect and the resultant increase in injury can be abrogated by passive immunization against IL-1β.[27] This implies that IL-1β has a direct influence on the severity of glomerular injury in this situation. Recently, injection of pharmacological doses of IL-1ra have been shown to reduce injury in rat models of nephrotoxic nephritis.[28–30] These studies emphasize the role of IL-1 in regulating injury and raise the question of whether IL-1ra is synthesized locally in glomerular inflammation and acts as a feedback regulator to limit its severity.

The purpose of this study was to examine the kinetics of local expression of IL-1ra gene in relation to IL-1β gene during the course of antibody-mediated glomerulonephritis in rats. We chose experimental methods to avoid *ex vivo* culture of tissues, so that the findings would reflect the kinetics of *in vivo* cytokine expression more accurately. This has enabled us to compare the relative levels of both mRNAs in inflamed glomeruli and in control tissues and to relate the findings to events within the glomerulus. The results show coordinated expression of IL-1ra and IL-1β genes in glomeruli after induction of accelerated nephrotoxic nephritis. In lungs and spleens of the same rats, increase of IL-1ra mRNA level was more substantial than that of IL-1β during induction of glomerulonephritis.

Materials and Methods

Reagents

Rabbit antiserum to rat glomerular basement membrane (GBM) was prepared as described previously[31] and was free from endotoxin as assessed by *Limulus* amoebocyte lysate assay (lower limit of detection: 5 pg/ml) using a test kit (Kabi Vitrim, Uxbridge, UK).

The cDNA probe for rat IL-1β was supplied by Dr. Alan Shaw (Glaxo Institute of Molecular Biology). The cDNA probe for rat IL-1ra was prepared locally by polymerase chain reaction (PCR) amplification of exon 4 coding region of rat IL-1ra sequence.[32] Using specific antisense primer, first strand cDNA was produced locally by reverse transcription of splenic RNA of rats injected with 0.25 μg LPS (from *Salmonella typhimurium,* Sigma, St. Louis, MO) 4 hours previously. The resulting cDNA was amplified by 30 cycles of PCR with 1 minute at 94 C (denaturation), 1 minute at 55 C (annealing), and 1 minute at 72 C (extension). The sequence for sense primer was GAG GTCGAC ATC ACT GAT CTG and that for antisense primer was TTG GAA TTC CTG GAA GTA GAA corresponding to positions 322–342 and 534–514 of the published sequence[32] with modification (as underlined) for *Hin*cII and *Eco*RI restriction sites, respectively. The cDNA probe for human tubulin kα1, which was used as a control, was a 1.6-kb insert subcloned into pSP64 plasmid.[33]

Induction of Nephritis

Male Sprague-Dawley rats (weight 194–248 g) were immunized by subcutaneous injection of 1 mg normal rabbit IgG (Sigma) in Freund's complete adjuvant (Sigma). Seven days later, they were injected with 1.5 ml of nephrotoxic serum intravenously via dorsal penile vein. Urine, plasma, and serum were collected before injection of nephrotoxic serum and at 4, 6, 24, 48, and 96 hours thereafter. Albuminuria was quantified by immunoelectrophoresis.[26] Endogenous creatinine clearance (CCl) was calculated from plasma and urinary measurements by using a sensitive colorimetric assay (Beckman autoanalyzer) which is specific for creatinine and subject to minimal interference from endogenous noncreatinine chromogens.[34] Urinary protein excretion was assessed first by 24-hour albuminuria and then by the urinary albumin/creatinine ratio [U(al/c) mg/mg] in timed urine collections. This enabled comparisons to be made between results from rats killed at 4 and 6 hours with those killed at later time points.

Morphology

Groups of 5 or 6 rats were studied at each time point. They were anesthetized with ether before the kidneys were perfused *in situ* with 50 ml of phosphate-buffered saline at room temperature, followed by 10 ml of ice-cold phosphate-buffered saline. Samples of renal tissue were snap-frozen in liquid nitrogen for immunohistochemistry or were fixed in formalin for light microscopy as previously described.[26] Neutrophils were counted in chloroacetate esterase-stained sections by counting 50 consecutive glomeruli[26]; monocytes were identified in frozen sections by indirect immunoperoxidase staining with the ED1 monoclonal antibody (Serotec, Oxford, UK).[35]

Collection of Tissues for mRNA Studies

The remaining kidney tissue was used to purify glomeruli by differential sieving through meshes of 250 μ, 150 μ, and 63 μ, washing continuously with ice-cold

phosphate-buffered saline. Immediately after purification, the glomeruli were suspended in 4 mol/L guanidine thiocyanate solution and homogenized at 24,000 rpm using Ika-ultra-Turbax T25 (Janke and Kunkel, Staufen). Total RNA was purified from the homogenate by the cesium chloride gradient ultracentrifugation method.[36] RNA was purified from lungs and spleens of the same rats for comparative studies.

Northern Analysis

Total RNA was quantified with spectrophotometry at 260 nm and the purity assessed from the ratio of absorption at 260 nm to that at 280 nm. Equal amounts of RNA were electrophoresed on a 3.2% formaldehyde, 1X MOPS, 1% agarose gel and transferred to GeneScreen plus (New England Nuclear, Boston, MA), by capillary blot using 10X SSC solution (1X SSC: 15 mmol/L trisodium citrate, 150 mmol/L sodium chloride). Filters were prehybridized for 16 hours at 42 C with 200 μg/ml denatured salmon sperm DNA (Sigma) in 50% formamide, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, 1 mol/L sodium chloride. Filters were hybridized overnight at 42 C with Klenow DNA polymerase-labeled ^{32}P-CTP cDNA probes[37] in the same prehybridization. They were washed with 1% SDS, 1X SSC solution, followed by 1% SDS, 0.1X SSC at 42 C. In some cases, additional washes with 1% SDS, 0.01–0.1X SSC were needed to reduce background radioactivity. The filters were then exposed to X-OMAT Kodak film with intensifier screen at 20 C or −70 C for various times up to 14 days. Multiple exposure times were used to ensure optimal conditions for densitometry. For IL-1β mRNA, autoradiographs were exposed for 4 days and 14 days at −70 C. For IL-1ra mRNA, autoradiographs were exposed for 1 day, 3 days, and 14 days at −70 C. For tubulin mRNA, autoradiographs were exposed for 1 day at −70 C and 20 C. A standard positive control of RNA purified from the spleen of a rat injected with 0.25 μg of endotoxin 4 hours previously was included on all filters so that the results could be compared directly. Filters were stripped of radioactivity by washing in boiling 0.01% SDS, 0.01X SSC solution before repeat hybridization with another cDNA probe. Degree of hybridization was assessed by scanning optimally exposed autoradiographs with a Chromoscan 3 densitometer (Joye Loebl, Gateshead, UK). Differences in RNA loading were corrected by reference to hybridization of the tubulin probe using the formula: IL-1β or IL-1ra mRNA/tubulin mRNA. These results were then expressed as a percentage of a standard positive control.

In Situ *Hybridization*

In situ hybridization for IL-1β and IL-1ra mRNA were performed using digoxigenin-labeled cDNA probes on formalin-fixed renal tissue from two rats at each time point. The rat IL-1β cDNA probe was nick-translated with digoxigenin-labeled dUTP as previously described for biotinylation.[38] A single-strand rat IL-1ra cDNA probe was generated by PCR amplification[39] of exon 4 coding region of rat IL-1ra sequence[32] using the antisense primer only for antisense probe, and sense primer for sense probe. Thirty cycles of PCR were performed consisting of 1 minute at 94 C (denaturation), 1 minute at 55 C (annealing) and 1 minute at 72 C (extension).

The method for *in situ* hybridization was modified from that described by Fleming and colleagues.[40] Formalin-fixed and paraffin-embedded renal tissue was cut at 3 μ and floated onto silane-coated slides. The slides were baked at 80 C for 30 minutes and dewaxed by immersion in Citroclear solution for 3 X 5 minutes and in acetone for 5 minutes. The sections were digested for 30 minutes with 10 μg/ml proteinase K (Sigma), then washed three times in double-distilled autoclaved water and immersed in acetone for 3 minutes before being washed an additional three times. Then, they were denatured in 70% formamide at 37 C for 30 minutes and air-dried. The sections were hybridized with denatured cDNA probe in 50% deionized formamide, 10% dextran sulfate, 0.05% polyvinylpyrrolidone, 0.05% SDS, herring sperm DNA 500 μg/ml, 2X SET: 0.3 mol/L NaCl, 2 mmol/L EDTA, 0.05 mol/L Tris HCl, pH 7.4, under sealed coverslips and incubated in a moisture chamber at 37 C for 17 to 24 hours. After being washed in 50% formamide, 20% 10X SET, 0.5% Triton X-100 at 37 C for 3 X 10 minutes and blocked in 15% nonfat milk (in 0.1 mol/L Tris, 0.1 mol/L NaCl, 0.02 mol/L $MgCl_2$, pH 7.5) at 37 C for 20 minutes in a moisture chamber, with brief rinses in buffer (0.1 mol/L Tris, 0.1 mol/L NaCl, 0.02 mol/L $MgCl_2$, pH 7.5), the sections were incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase (Boehringer) at the dilution of 1:750 for 30 minutes at 37 C. Further washing followed, in 0.1 mol/L Tris, 0.1 mol/L NaCl, 0.02 mol/L $MgCl_2$, pH 7.5, first for 3 X 5 minutes with 0.5% Triton X-100, then another 3 X 5 minutes without Triton. The sections were washed a final three times in 0.1 mol/L Tris, 0.1 mol/L NaCl, 0.1 mol/L $MgCl_2$, pH 9.0 at room temperature before being developed in nitro blue tetrazolium and bromochloroindolyl phosphate in the dark for 3 to 15 hours. Negative controls were performed on renal tissue sections, which were pretreated with RNase. As negative control for IL-1β cDNA probe,

nick-translated cDNA from pGEM plasmid without the insert sequence was used. Last, as negative control for IL-1ra antisense cDNA probe, a single-strand sense cDNA probe was used.

Results

Glomerulonephritis

Groups of 5 or 6 rats were studied at time points between 0 and 96 hours after induction of nephritis. All rats developed acute glomerulonephritis with albuminuria that increased progressively from 4 to 96 hours, as demonstrated by urinary albumin/creatinine ratios and 24-hour albumin excretion (Table 1). Despite severity of the proteinuria, there was no significant change in creatinine clearance during the experiment (Table 1).

The glomeruli were markedly hypercellular 4 hours after induction of nephritis (Figure 1), and many contained capillary thrombi by 24 hours. Severe focal and segmental necrotizing glomerulonephritis developed between 48 and 96 hours. The chloroacetate esterase stain showed a rapid but transient neutrophil influx, maximal at 4 and 6 hours (Table 1). Results with monoclonal antibody ED1 showed a slower and more sustained monocyte influx, reaching a plateau level from 24 to 96 hours (Table 1). In summary, the protocol resulted in a model of acute nephritis with sequential influx of neutrophils and monocytes and evidence of increasingly severe glomerular injury.

Steady-State Glomerular mRNA Level

Steady-state mRNA levels for IL-1β and IL-1ra were quantified from Northern blot using 26 μg of pooled total RNA purified from three rats at each time point. As a positive control for hybridization, 6 μg of splenic RNA extracted from a rat 4 hours after injection of 0.25 μg of LPS was used. IL-1β and IL-1ra could not be detected in Northern blots of glomerular RNA from normal or immunized rats. Both species of mRNA were readily detectable after injection of nephrotoxic antibodies with maximal hybridization between 4 and 6 hours. Thereafter, levels decreased, although both mRNAs were still detectable at 96 hours (Figure 2). Hybridization to tubulin confirmed that the change in mRNA level was not due to loading differences. Quantitation of hybridization by densitometry, after normalization for tubulin mRNA level, confirmed obvious differences in the kinetics of the IL-1β and IL-1ra responses (Table 2). Both mRNAs increased rapidly, but IL-1ra expression was more sustained and was at 67% and 30% of peak values at 24 and 48 hours, respectively. In comparison, the IL-1β mRNA was 25% and 10% of its peak values at 24 and 48 hours, respectively. Two further sets of Northern blots were performed using glomerular RNA from different individual rats at the same time points (data not shown). They confirmed the kinetics of IL-1β and IL-1ra gene expression and the relative differences between them.

In Situ *Hybridization in Glomeruli*

Two rats were examined at each time point. There was no detectable hybridization of IL-1β or IL-1ra probes to kidney sections from normal rats or those studied immediately before induction of nephritis (Figures 4 and 5). However, IL-1β mRNA (Figure 4) expressed intensively in glomeruli from rats killed at 4 and 6 hours (only results at 4 hours was shown). All glomeruli were involved and the pattern of hybridization was diffuse. There was also a few strongly positive cells. In addition, positively stained parietal epithelium can be seen adjacent to proximal tubules at 4 hours. Because of the diffuse expression of IL-1β mRNA, counting of individual positive cells was not

Table 1. *Excretion of Creatinine and Albumin and Glomerular Leukocyte Infiltration in Accelerated Nephrotoxic Nephritis**

		Hours after Induction					
	Normal ($n = 5$)	0 ($n = 5$)	4 ($n = 5$)	6 ($n = 6$)	24 ($n = 5$)	48 ($n = 5$)	96 ($n = 6$)
U (al/c)	0.12 ± 0.12	0.12 ± 0.08	11.1 ± 8.4	19.3 ± 4.4	41.5 ± 14.6	79.9 ± 26.6	126 ± 51
Albuminuria (mg/24 h)	0.7 ± 0.7	0.6 ± 0.4	N/C	N/C	321 ± 100	565 ± 155	596 ± 46
CCl (ml/min)	0.64 ± 0.07	0.72 ± 0.09	N/C	N/C	0.69 ± 0.06	0.71 ± 0.08	0.70 ± 0.06
Neutrophils/glomerular section	0.03 ± 0.03	0.08 ± 0.07	6.1 ± 0.6	5.5 ± 0.8	0.9 ± 0.3	0.5 ± 0.2	0.2 ± 0.04
Monocytes/glomerular section	0.6 ± 0.01	0.7 ± 0.08	4.2 ± 0.4	3.9 ± 0.7	8.8 ± 1.2	9.6 ± 0.3	6.7 ± 1.8

* Results are expressed as means ± SE. N/C: values are not calculated because 24-hour urine cannot be collected for 4- and 6-hour time point. Neutrophils and monocytes per glomerular section was calculated from the mean of 50 consecutive glomerular sections from each rat.

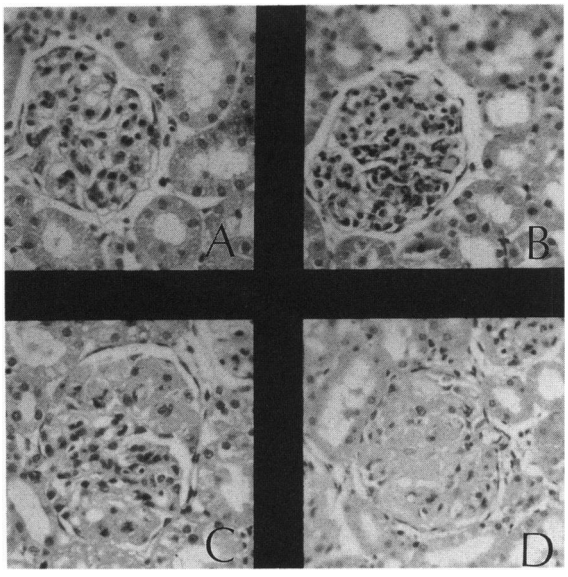

Figure 1. *Renal tissue from preimmunized rats showed normal morphology* (**A**). *Induction of nephritis results in hypercellularity in glomeruli at 4 hours* (**B**) *and glomerular capillary thrombosis by 24 hours* (**C**). *Severe focal and segmental glomerular necrosis developed by 96 hours* (**D**). *Sections were stained with hematoxylin and eosin* (×*120*).

possible. The overall pattern is compatible with multiple cell types in the glomeruli expressing IL-1β mRNA, although cell markers will be necessary to characterize the nature of these positive cells. There was no hybridization when nick-translated pGEM cDNA was used as negative control for renal tissues from all the time points. *In situ* hybridization with the IL-1ra probe was also obvious after induction of nephritis but was more discretely localized and limited to individually positive cells at most time points (Figure 5). The number of positive cells was maximal between 6 and 24 hours (Table 3) when there was an average of 18.8 and 13.3 cells/glomerular section, respectively; thereafter the number of positive cells decreased to a third of this value by 96 hours. Sense IL-1ra probe (negative control) did not hybridize to renal tissues from any time point.

Steady-State Level of IL-1β and IL-1ra mRNA in Spleen and Lung

The levels of IL-1β and IL-1ra mRNA were measured to compare the glomerular results with those of uninflamed tissues. For Northern blotting (data not shown), 26 μg of total RNA from the spleens and lungs (which were pooled from three rats) was used at each time point. Identical positive control (6 μg of splenic RNA from a LPS-injected rat) was used. The results of densitometric analysis of the Northern blots are compared with those from glomerular RNA and summarized in Figure 3. A small amount of IL-1β mRNA was detectable in spleens and lungs from normal rats and in those from rats immunized with rabbit IgG. There was an increase in IL-1β mRNA in both tissues immediately after induction of nephritis, but the increase was much less than that seen in glomeruli. The level returned to baseline by 24 hours for lungs and by 48 hours for spleens. IL-1ra mRNA was just detectable in lungs from rats before induction of glomerulonephritis, but not in spleens. The level of IL-1ra mRNA increased substantially in both tissues after injection of the anti-GBM antibodies. At 4 hours, the splenic level of IL-1ra mRNA was even higher than the glomerular level.

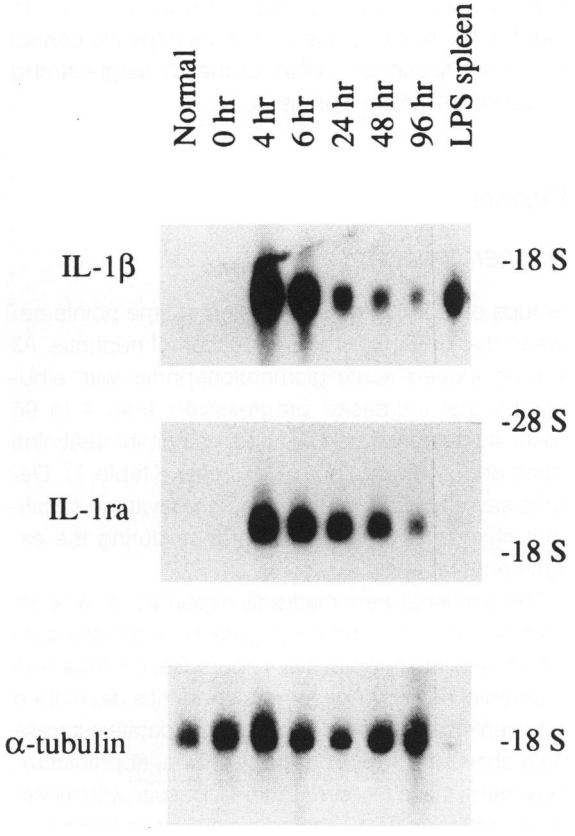

Figure 2. *Induction of nephritis caused increase IL-1β and IL-1ra mRNA expression (maximal at 4 and 6 hours). Tubulin mRNA level showed the changes in levels of IL-1β and IL-1ra mRNA was not due to loading differences. In the Northern blot, 26 μg of total RNA extracted from glomeruli pooled from 3 rats were used for each glomerular sample. Splenic RNA (6 μg) from an LPS-injected rat (LPS spleen) was used as a positive control. Autoradiographic exposures were 14 days for IL-1β, 3 days for IL-1ra, and 1 day for tubulin mRNA at −70 C. Densitometric analysis of this Northern blot is shown in Table 2. Results were representative of 3 Northern blots showing similar results.*

Discussion

IL-1 has a crucial role in modulating the intensity of acute inflammation,[26,41] and it is increasingly appar-

Table 2. *Densitometric Analysis of Glomerular mRNA Levels in Accelerated Nephrotoxic Nephritis**

	Normal	Hours after Induction						Positive Control
		0	4	6	24	48	96	
IL-1β/tubulin	0	0	35	40	10	4	3	100
IL-1ra/tubulin	0	0	371	347	248	111	52	100

* Densitometric values are derived from the Northern blot in Figure 2 and expressed as percentage of the positive control (6 μg splenic RNA from a LPS-injected rat). Each glomerular RNA sample was extracted from glomeruli pooled from three rats.

Table 3. *Number of Cells/Glomerular Section Expressing IL-1ra mRNA in Accelerated Nephrotoxic Nephritis**

Normal	Hours after Induction					
	0	4	6	24	48	96
0	0	11.6	21.0	10.4	6.9	3.8
0	0	14.2	16.7	16.2	7.2	7.6

* *In situ* hybridization was performed on two rats killed at each time point. Results from each of these animals were shown in the table as positive cells/glomerular section.

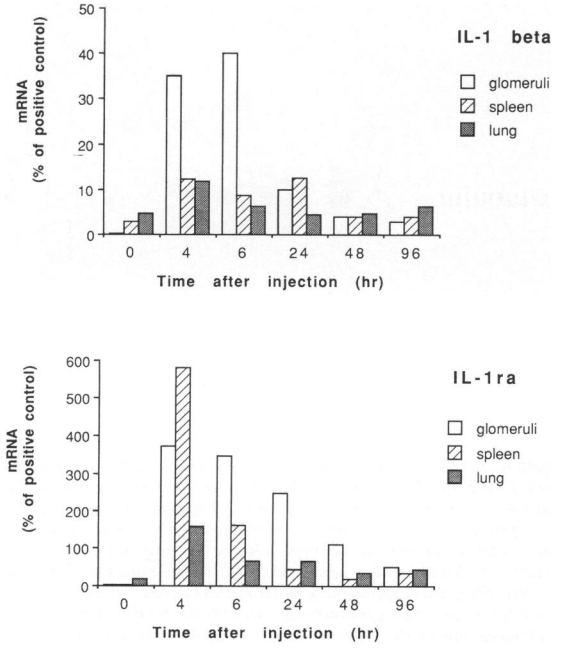

Figure 3. *Following induction of nephritis, increase of IL-1β and IL-1ra peaked at 4 and 6 hours, subsequently IL-1β mRNA reduced by a greater proportion than IL-1ra mRNA. The change of IL-1β mRNA level in lung and spleen was less than that of glomeruli. Increase of IL-1ra mRNA level was more substantial in spleen and lung. At 4 hours, the splenic IL-1ra mRNA level was even higher than the glomerular level. The densitometric analysis of Northern blot for IL-1β mRNA, IL-1ra mRNA was normalized to the tubulin mRNA level for loading differences. The results were from analysis of RNA extracted from tissues pooled from 3 rats at each time point. Results were expressed as percentage of the same positive control (6 μg of splenic RNA from an LPS-injected rat).*

ent that its proinflammatory effects are modified by the natural inhibitor IL-1ra.[13] *In vitro* studies have shown that the effective concentration of IL-1 depends on the molar ratio of agonist to antagonist, as both have high affinity for IL-1 receptors.[14] This relationship is illustrated well by experiments showing that IL-1ra inhibits the capacity of IL-1α to stimulate human mesangial cells to synthesize the chemokine IL-8.[42] Pharmacological doses of IL-1ra modulate the effects of IL-1 *in vivo* and protect rabbits and mice from lethal injections of bacterial endotoxin.[43,44] There are few data about the synthesis of IL-1ra at sites of inflammation *in vivo*, except after local or systemic injections of endotoxin.[17–19,45] However, clinical studies in Lyme arthritis,[20] rheumatoid arthritis,[46] and ulcerative colitis[47] demonstrated co-expression of IL-1β and IL-1ra in synovial fluid and synovial and mucosal biopsies, but the kinetics of the responses could not be studied.

Here we demonstrate coordinated expression of IL-1ra and IL-1β genes immediately after the induction of the antibody-mediated glomerulonphritis, with steady state of mRNA levels of both reaching maximal values within 4 to 6 hours after injection of anti-GBM antibodies. The kinetics of glomerular IL-1ra and IL-1β mRNA were different at later time points, with IL-1ra mRNA more sustained. In addition, the upregulation of IL-1ra was not confined to glomeruli only, but also substantially increased in lung and spleens after induction of nephritis, but the changes in IL-1β in the lung and spleen were small.

This model of antibody-mediated organ-specific injury also provides the opportunity to compare IL-1ra and IL-1β in the inflamed organ with other tissues, which are spared from injury. This experimental model has the additional advantage of inducing inflammatory injury in the absence of bacterial products. The experimental approach avoided the use of *in vitro* glomerular culture techniques, which provide data about the capacity of glomerular cells to produce particular cytokines rather than whether they were producing them *in vivo*. Instead we assessed the kinetics of local IL-1ra and IL-1β network, using Northern blot analysis

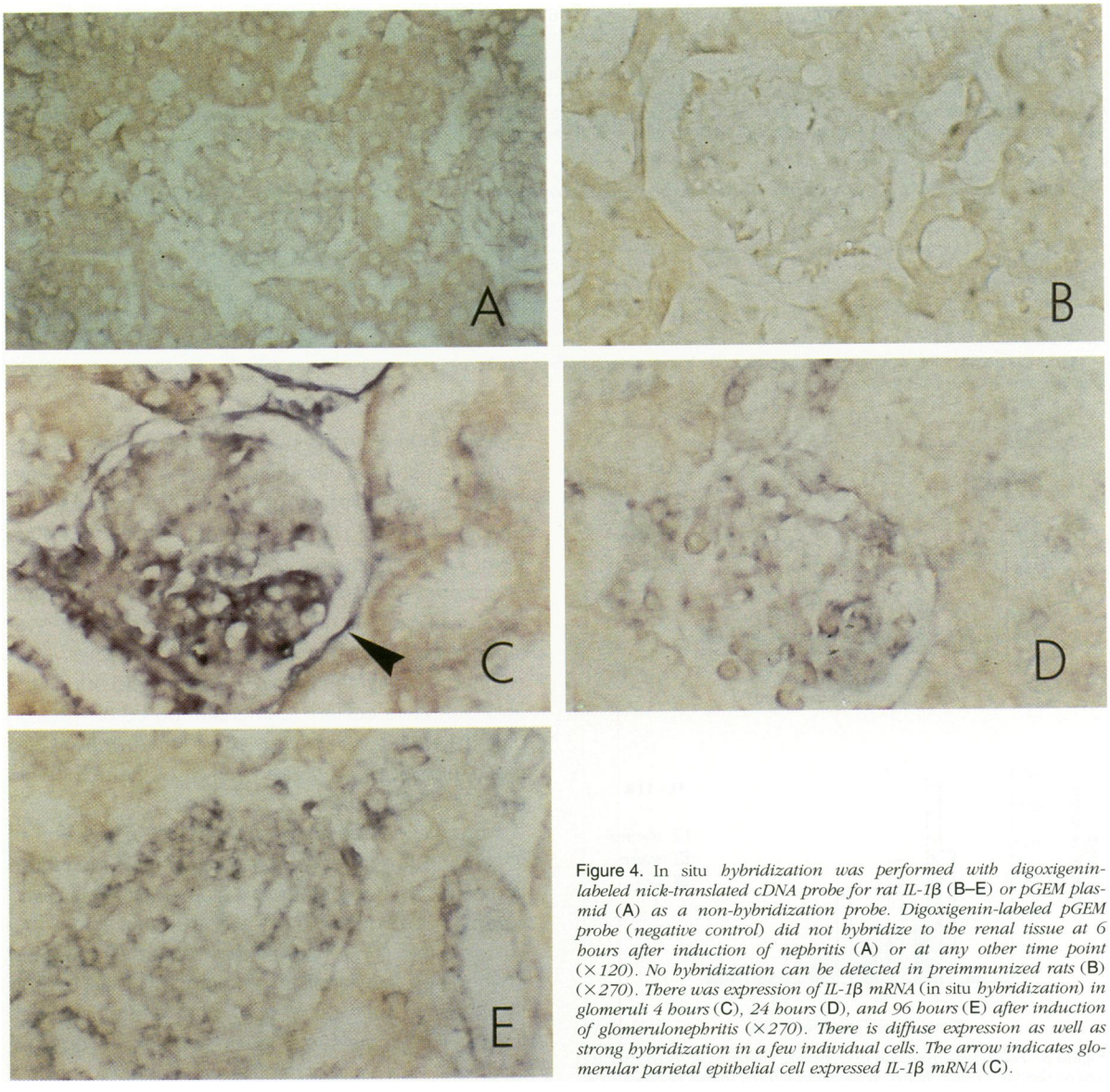

Figure 4. In situ *hybridization was performed with digoxigenin-labeled nick-translated cDNA probe for rat IL-1β* (B–E) *or pGEM plasmid* (A) *as a non-hybridization probe. Digoxigenin-labeled pGEM probe (negative control) did not hybridize to the renal tissue at 6 hours after induction of nephritis* (A) *or at any other time point* (×120). *No hybridization can be detected in preimmunized rats* (B) (×270). *There was expression of IL-1β mRNA* (in situ *hybridization) in glomeruli 4 hours* (C), *24 hours* (D), *and 96 hours* (E) *after induction of glomerulonephritis* (×270). *There is diffuse expression as well as strong hybridization in a few individual cells. The arrow indicates glomerular parietal epithelial cell expressed IL-1β mRNA* (C).

as a semi-quantitative approach, because bioassay of tissue extract will not be suitable to differentiate the production the IL-1β and IL-1ra, and specific immunoassays for them are not yet available; this precludes measurement of expressed protein at present.

With regard to the physiological significance of the finding, IL-1 bioactivity was detected when glomeruli from this model were cultured *ex vivo*,[24] although such an approach did not allow detailed kinetics to be assessed. Second, infusion of recombinant IL-1ra has been shown to reduce glomerular injury in accelerated nephrotoxic nephritis in the rat,[28] which demonstrates that a sufficient concentration of IL-1ra can reduce glomerular inflammation in this experimental model. The importance of glomerular IL-1ra in glomerulonephritis has been further supported by the effect of intervention treatments in an endotoxin-enhanced model of heterologous phase nephrotoxic nephritis, in which infusions of recombinant IL-6,[48] IL-1ra, and soluble IL-1 receptor[30] all caused a substantial reduction of glomerular IL-β mRNA level but only moderate reduction of IL-1ra mRNA level. These changes increased the proportion of glomerular gene expression of IL-1ra to that of IL-1β and was associated with reduction of neutrophil infiltration and glomerular injury.

It was not surprising that increased glomerular expression of IL-1ra was insufficient to prevent tissue

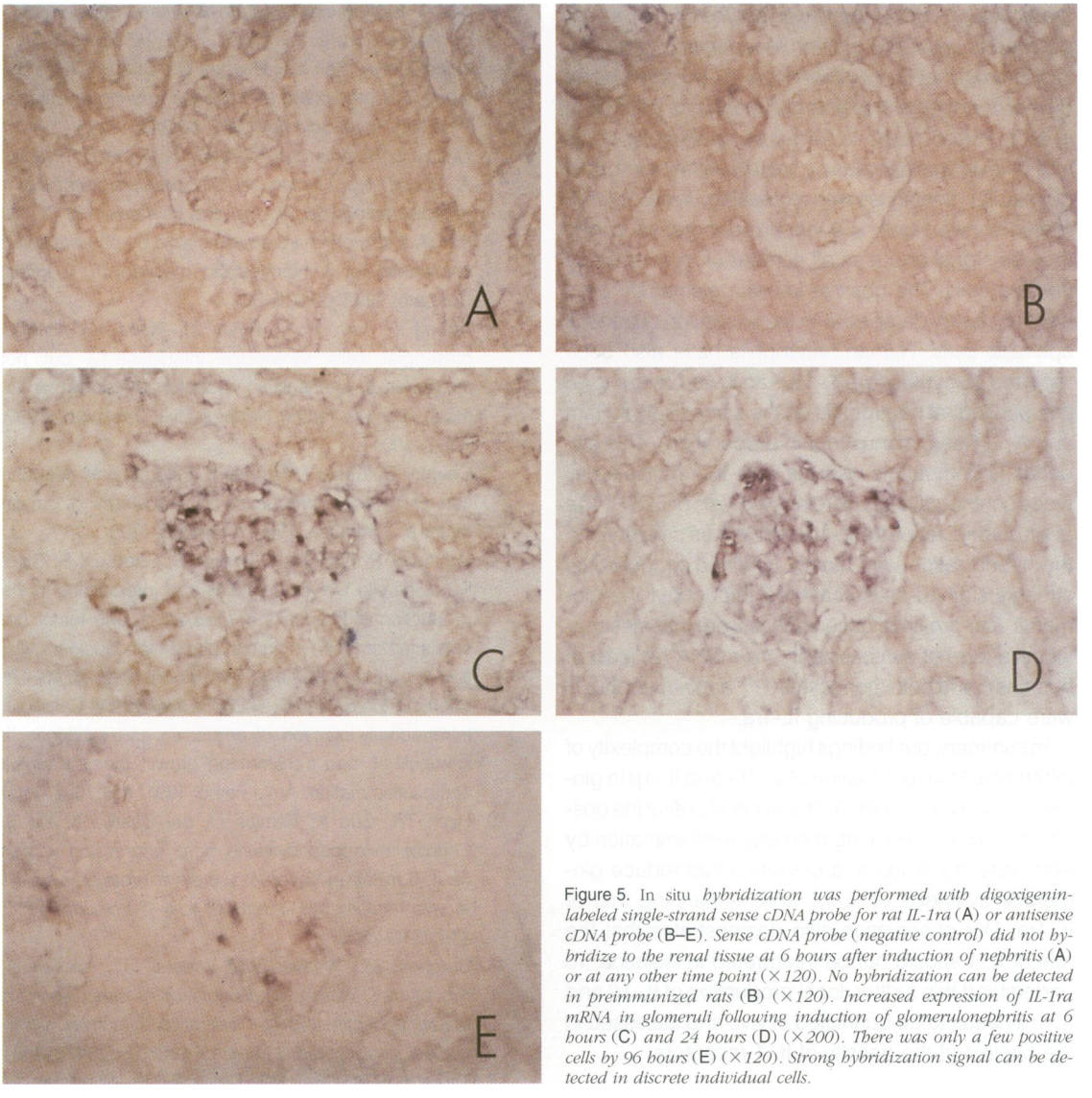

Figure 5. In situ *hybridization was performed with digoxigenin-labeled single-strand sense cDNA probe for rat IL-1ra* (A) *or antisense cDNA probe* (B–E). *Sense cDNA probe (negative control) did not hybridize to the renal tissue at 6 hours after induction of nephritis* (A) *or at any other time point* (×120). *No hybridization can be detected in preimmunized rats* (B) (×120). *Increased expression of IL-1ra mRNA in glomeruli following induction of glomerulonephritis at 6 hours* (C) *and 24 hours* (D) (×200). *There was only a few positive cells by 96 hours* (E) (×120). *Strong hybridization signal can be detected in discrete individual cells.*

injury completely. Similar observations have been made in lethal *Escherichia coli* septicemia in the baboon, where the peak plasma level of IL-1ra is eightfold higher than that of IL-1β[18] and infusion of a pharmacological dose of IL-1ra improves the survival.[49] It has been shown that 100–1000 molar excess of IL-1ra was required to inhibit the effects of injected IL-1 *in vivo*[14] and that IL-1ra modulates inflammation *in vivo*.[50] In our model, the glomerular IL-1ra mRNA levels relative to that of IL-1β increased progressively over the first 2 days, from 10-fold more abundant at 4 and 6 hours to 20-fold more abundant at later time points. However, the physiological contribution of IL-1ra in the glomeruli depends on the effective concentration in the microenvironment, and so considerable caution must be exercised when extrapolating results from the whole glomeruli. Future experiments will help to clarify the issue further when neutralizing antibody to rat IL-1ra is available. In our model, substantial increase of IL-1ra mRNA and small IL-1β response in lungs and spleens are notable and could be part of the protective response to cytokine released from inflamed tissue elsewhere in the body or to activated circulating leukocytes. The differences in balance of IL-1ra and IL-β gene expression could be expected to protect lungs and spleens from tissue injury and thus focus inflammation to the site of antibody deposition. To date, there are no data bearing

on the cellular basis of the differences in gene expression between these organs.

Monocytes and activated macrophages are generally thought to be the major source of IL-1 in experimental anti-GBM glomerulonephritis,[51] and so it is of interest that IL-1β mRNA level in our experiments did not correlate with glomerular monocyte infiltration; in fact, from 6 hours to 24 hours, glomerular IL-1β mRNA level was decreasing rapidly as the number of glomerular monocytes was increasing. *In situ* hybridization showed both a generalized increase in glomerular signal and a small number of more strongly positive cells. The positive intrinsic glomerular cells have not been identified with the exception of parietal epithelial cells. Human neutrophils have also been reported to synthesize IL-1β,[3] and we have shown that purified rat neutrophils do the same (F. W. K. Tam and A. J. Rees, manuscript in preparation). This evidence is compatible with the view that multiple cell types contribute to glomerular synthesis of IL-1.[24] The *in situ* hybridization for IL-1ra mRNA was different and the hybridization was mainly confined to individual strongly stained cells scattered throughout the glomeruli. Our previous observations were that mesangial cells did not synthesize IL-1ra mRNA (at least in humans)[6] and both monocytes[15,16] and neutrophils[19] were capable of producing IL-1ra.

In summary, our findings highlight the complexity of differential local production of IL-1ra and IL-1β in glomeruli, lungs, and spleen. These results raise the possibility of downregulating glomerular inflammation by administering drugs and cytokines that reduce glomerular synthesis of IL-1β while enhancing that of IL-1ra. Our observation also demonstrates that the role of IL-1 should not be interpreted in isolation. To understand this further, local production of IL-1β and IL-1ra needs to be quantified *in vivo* and assessed in the context of the density of IL-1 receptors.

Acknowledgments

We are grateful to Dr. Alan Shaw (Glaxo Institute of Molecular Biology) for supplying the cDNA probe for rat IL-1β and to Jeremy Beacham, who kindly performed the creatinine measurement.

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