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Interleukin-1β gene expression in experimental glomerulonephritis in the rat: an in-situ hybridization study

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Summary. To investigate the role of interleukin-1β (IL-1β) in glomerulonephritis we studied the presence of IL-1β by in-situ hybridization in in-situ immune complex glomerulonephritis in the rat. Glomerulonephritis was induced in preimmunized rats by unilateral renal perfusion with cationized human IgG. In-situ hybridization was performed on frozen sections with a battery of four 30mer oligonucleotide DNA probes 3′ end labelled with ^{35}S-dATP. IL-1β mRNA was detectable in nephritic glomeruli at 6 and 24 hours after induction of glomerulonephritis. Signal was maximal at 48 hours and markedly reduced by 4 days. The peak of IL-1β transcription coincided with the major monocyte influx into glomeruli consistent with a role for IL-1β as a mediator of glomerular hypercellularity.

Keywords: glomerulonephritis, interleukin-1, in-situ hybridization

Interleukin-1 (IL-1) is an important mediator with a wide range of proinflammatory and immunological effects (reviewed in Dinarello & Wolff 1993). It was first described as a product of mononuclear phagocytes but is now known to be released by many cell types including mesangial cells (Lovett *et al.* 1985; Werber *et al.* 1987). There are two forms of IL-1, α and β, which are the products of separate genes. They have different amino acid sequences but act through the same cell surface receptors and share biological activities. Most IL-1α remains in the cytosol of cells or is associated with the cell membrane where it may act as a paracrine messenger to adjacent cells. In contrast, a considerable amount of IL-1β is released by cells into the extracellular space and the circulation.

IL-1 acts systemically to induce fever, the acute phase response and neutrophilia and locally to stimulate neutrophil chemotaxis, activate endothelial cells and induce a range of other cytokines including interleukins 1, 2, 6, 8 and tumour necrosis factor. It acts on mesangial cells to enhance their proliferation (Lovett *et al.* 1983), and to stimulate eicosanoid production, collagen synthesis and production of a basement membrane degrading neutral protease (Sedor *et al.* 1992). Infusion of IL-1 has been shown to increase proteinuria in rat nephrotoxic nephritis (Isha *et al.* 1989) and administration of IL-1 receptor antagonist in accelerated nephrotoxic nephritis reduced proteinuria and glomerular hypercellularity (Lan *et al.* 1993).

The production of IL-1 has been examined in a number of forms of experimental glomerulonephritis. In rat accelerated nephrotoxic nephritis (Matsumoto & Hatano 1989) and passive autologous anti-glomerular basement membrane antibody induced glomerulonephritis in the rabbit (Tipping *et al.* 1991), IL-1 production has been demonstrated in the supernatants of isolated glomeruli. However, these experiments demonstrate production of

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IL-1 by glomeruli in culture but do not directly show IL-1 production *in vivo*. In a model of membranous glomerulonephritis in the rat, increased IL-1 mRNA was demonstrated in whole kidney (Werber *et al.* 1987) and in a model of lupus nephritis in the mouse there was increased IL-1β gene expression in renal cortex (Boswell *et al.* 1988a,b). These studies provide information about in-vivo gene expression but not about tissue localization. In acute aminonucleoside nephrosis, IL-1α has been shown in glomeruli immunohistochemically (Diamond & Pesek 1991). We have now studied a model of hypercellular immune complex glomerulonephritis in the rat and have examined IL-1β gene expression in kidneys by in-situ hybridization using oligonucleotide probes.

Materials and methods

Animals

Inbred male Lewis rats bred at St Mary's Hospital Medical School were used.

Experimental design

Unilateral in-situ immune complex glomerulonephritis was induced as previously described (Cook *et al.* 1989). Rats were immunized three times at monthly intervals with 1 mg human IgG emulsified in Freund's complete adjuvant and then the isolated left kidney was perfused with 400 μg cationized human IgG (pI > 9.7). Rats were killed at 6 hours ($n = 2$), 24 hours ($n = 6$), 2 days ($n = 6$) and 4 days ($n = 4$). Samples of renal cortex from nephritic left kidneys and normal right kidneys were frozen immediately in liquid nitrogen, embedded in Tissue Tek OCT compound (Raymond Lamb, London) and stored at -70°C until use. Cryostat sections were cut at 10 μm thickness and mounted on Vectabond coated slides (Vector Laboratories, Peterborough, UK). They were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) for 4 minutes. If not processed the same day, the slides were stored at -70°C until use. Tissue for light microscopy was fixed in formal saline, embedded in paraffin and stained with haematoxylin and eosin, and periodic acid–Schiff.

In-situ hybridization

The sequence of rat IL-1β was supplied by Glaxo, Geneva. Four 30-base synthetic oligonucleotide probes complementary to coding regions of this sequence were purchased from Oswel DNA Service, Edinburgh, UK. The sequences of these probes were as follows: Probe 1 (nucleotides 627–656): 5′-CTG-GGC-TCC-AAC-TGC-ATA-AGT-AGC-AAT-GAA-3′. Probe 2: (nucleotides 969–998): 5′-TTT-TGT-TGT-TCA-TCT-CGA-AGC-CTG-CAG-TGC-3′. Probe 3 (nucleotides 1224–1253): 5′-AAG-ACA-AAC-CGC-TTT-TCC-ATC-TTC-TTC-TTT-3′. Probe 4 (nucleotides 1273–1302): 5′-GAA-CTG-TGC-AGA-CTC-AAA-CTC-CAC-TTT-GGT-3′.

The oligonucleotides were 3′ end-labelled to a specific activity of between 1.0 and 2.0×10^7 c.p.m./pmol with ^{35}S-dATP and in-situ hybridization was carried out on cryostat sections as previously described (Taylor *et al.* 1992). Tissue sections were studied by use of a combined battery of all four antisense probes.

Specificity of in-situ hybridization was confirmed, in selected cases, by use of the following controls.

(1) Ribonuclease treatment of sections
Control slides were treated before hybridization with ribonuclease A 20μg/ml from bovine pancreas (R-9005, Sigma Chemical Co., Poole, UK) together with 80 units/ml ribonuclease T_1 (R-8251, Sigma) in 10 mM PBS for 30 minutes at 37°C.

(2) Competition with unlabelled probes
Hybridization was assessed after competition with either the unlabelled probes used at a 50-fold excess (350 ng/slide), which should abolish specific signal, or with a large excess (10 μg/slide of an unlabelled 'irrelevant' oligonucleotide of similar G + C composition (47%)) which should not affect specific hybridization signal.

(3) Slides were probed with an irrelevant 30-mer which had been 3′ tailed with ^{35}S-dATP.

For comparison of intensity of hybridization between kidneys a semi-quantitative scoring system was used. One section from each time point was selected and these sections were randomized; each glomerulus in the section was then given a score of 0, 1, 2 or 3 for the intensity of hybridization signal and a mean score was calculated for each section and then for each time point.

Results

Light microscopy

Rats developed a unilateral proliferative glomerulonephritis. They do not develop proteinuria since the left kidney stops producing urine almost completely during the acute phase of the disease (unpublished observations). We have previously described some of the glomerular changes in this model (Cook *et al.* 1989) using a method of enzymatic digestion which allowed quantitation of infiltrating leucocytes. This showed that at 24 hours there were 65 ± 22 mononuclear phagocytes per

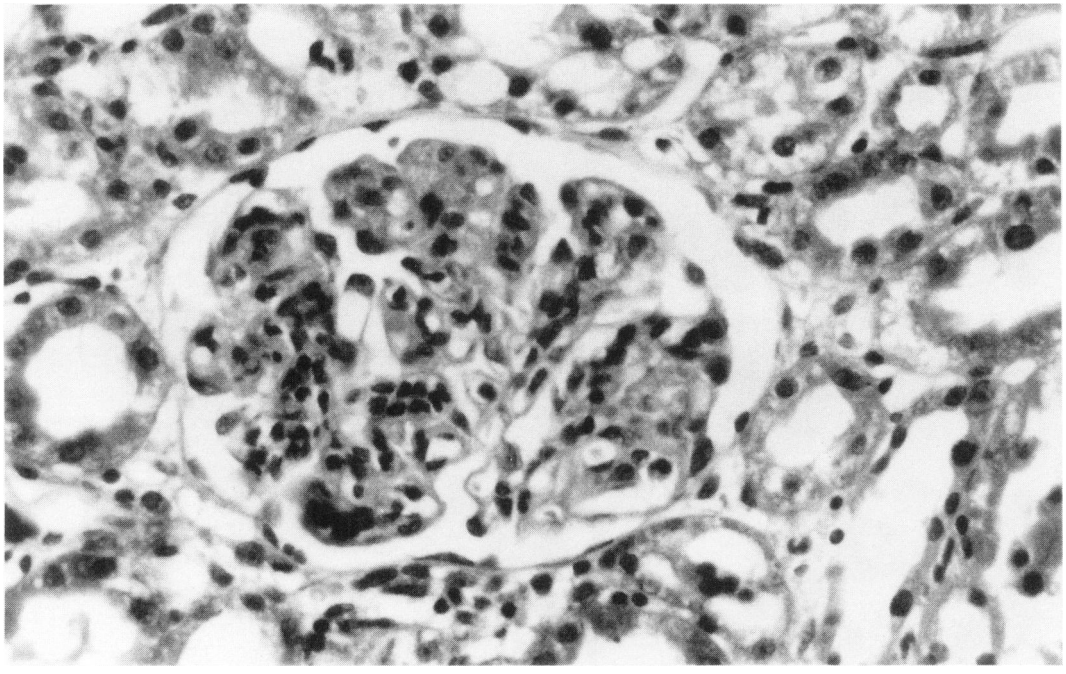


Figure 1. Glomerulus 48 hours after induction of glomerulonephritis. There is prominent focal hypercellularity. H & E. × 430.

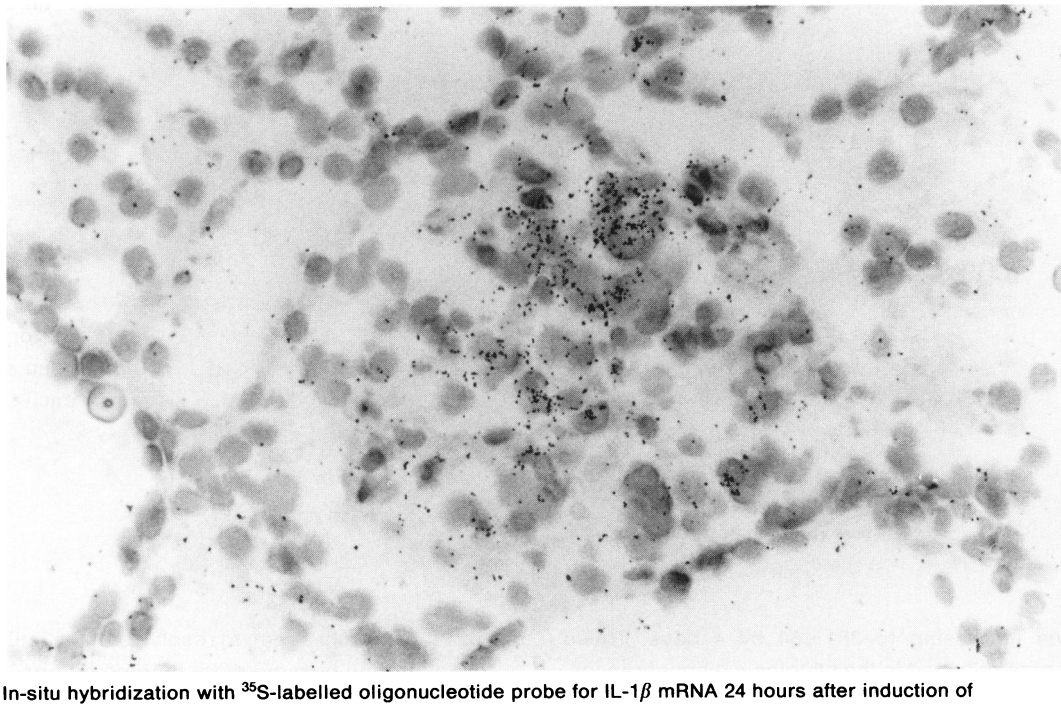
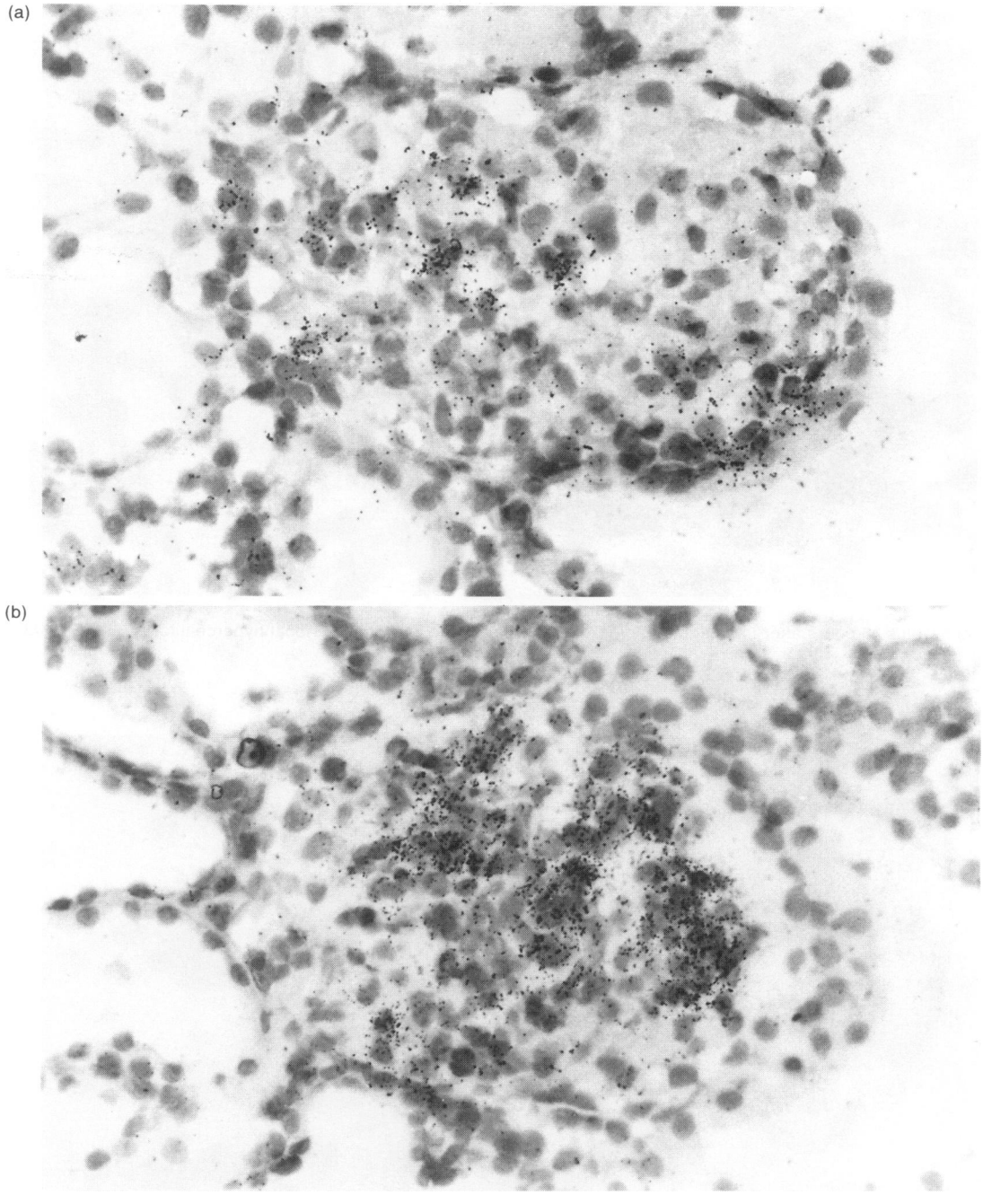


Figure 2. In-situ hybridization with ^{35}S-labelled oligonucleotide probe for IL-1β mRNA 24 hours after induction of glomerulonephritis. Signal is clearly localized to the glomerulus with a predominantly cytoplasmic distribution. Haematoxylin counterstain. × 400.



glomerulus increasing to 381 ± 64 by 4 days. These changes were reflected in the histological appearances. At 6 hours, glomeruli appeared mildly hypercellular with infiltrating neutrophils and mononuclear cells in capillary lumens. By 24 hours, many glomeruli showed capillary thrombi with some apparent loss of endothelial cell nuclei and persistent leucocyte infiltration. From day 2 to 4 the thrombi began to resolve and the inflammatory cell infiltration became more marked (Figure 1).

After in-situ hybridization no localization of signal was seen in kidneys from normal rats or in right non-nephritic kidneys from experimental rats at any of the time points studied. In nephritic kidneys a weak signal was present over glomeruli of kidneys isolated 6 and 24 hours (Figure

(c)

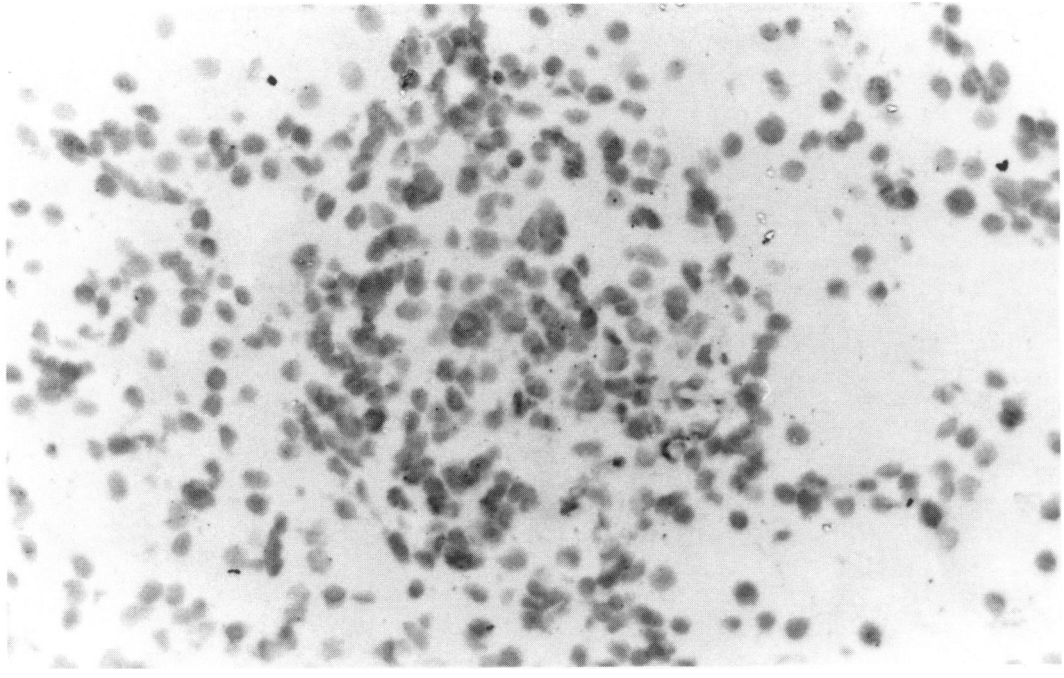


Figure 3. In-situ hybridization with ^{35}S-labelled oligonucleotide probe for IL-1β mRNA 48 hours after induction of glomerulonephritis. a, Signal is present over several cells in this mildly hypercellular glomerulus. Haematoxylin counterstain. × 475. b, Intense hybridization localized to hypercellular regions of the glomerulus. From the same section as a. Haematoxylin counterstain. × 405. c, Hybridization signal is absent after pretreatment with RNase. Haematoxylin counterstain. × 400.

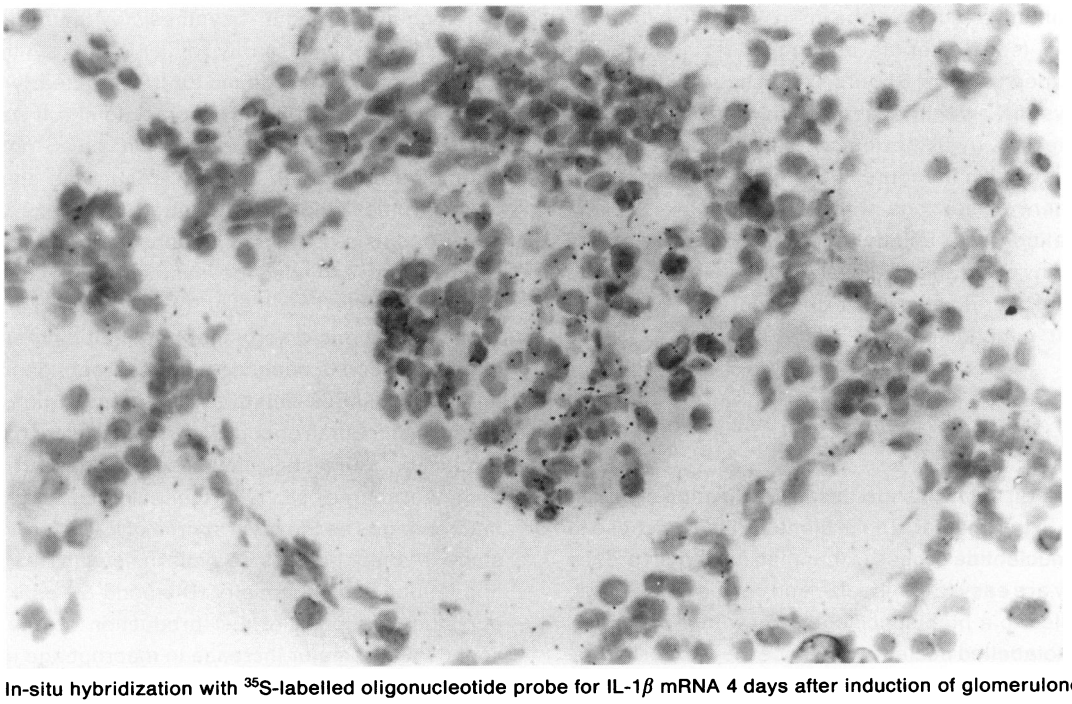


Figure 4. In-situ hybridization with ^{35}S-labelled oligonucleotide probe for IL-1β mRNA 4 days after induction of glomerulonephritis. A faint signal is still present in some glomeruli. Haematoxylin counterstain. × 400.

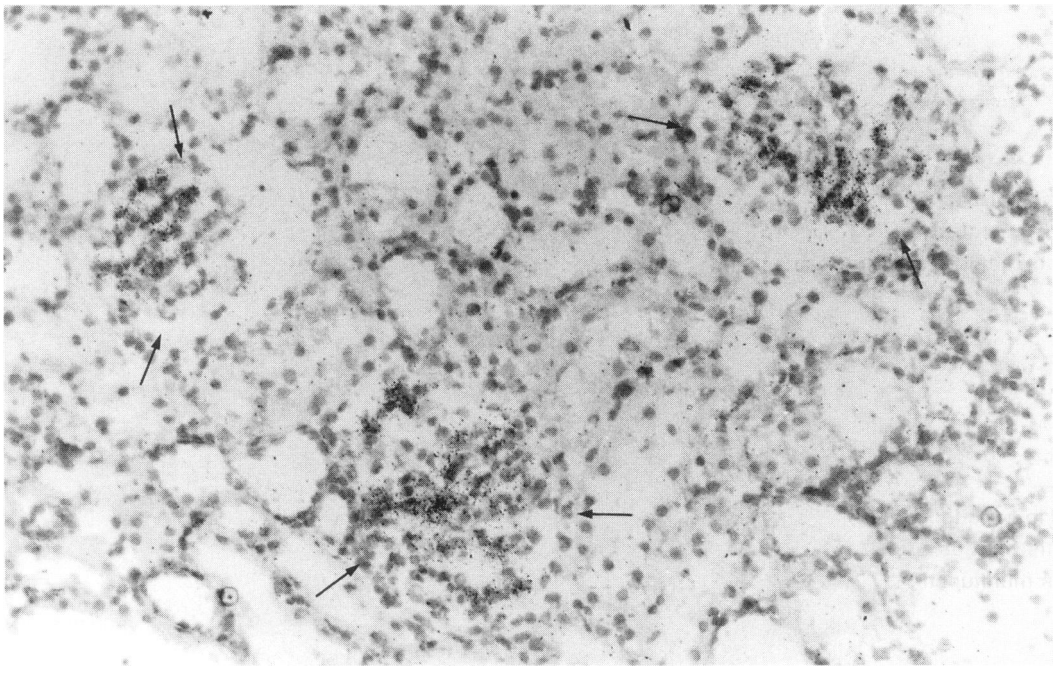


Figure 5. In-situ hybridization with ^{35}S-labelled oligonucleotide probe for IL-1β mRNA 48 hours after induction of glomerulonephritis. Three glomeruli are present in this low power view (arrows) and each shows hybridization signal. Haematoxylin counterstain. × 185.

2) after induction of glomerulonephritis. The intensity of hybridization was maximal at 2 days (Figure 3 a and b) and then diminished although some signal was still detectable in two of four rats at 4 days (Figure 4). Semiquantitative scoring of the intensity of the hybridization signal (see Materials and methods) showed the following mean scores: 6 hours, 0.8; 24 hours, 1.0; 2 days, 1.4; 4 days, 0.4. Within each section there was similar signal intensity over the majority of the glomeruli; an example is shown in Figure 5. Signal was abolished by pretreatment of sections with RNase (Figure 3c) and by competition with unlabelled IL-1β probe but not by competition with an 'irrelevant' oligonucleotide. No glomerular hybridization was detected with a labelled 'irrelevant' oligonucleotide.

Discussion

This is the first study to show tissue localization of mRNA for IL-1 in experimental glomerulonephritis. We chose to use oligonucleotide probes rather than full length probes since they are easy to synthesize and can be efficiently 3′ end labelled to a high specific activity with a polymeric tail of radiolabelled nucleotides. Since they are DNA they are considerably easier to handle than, for example, riboprobes. Because of their small size they have easy access to tissue RNA and we have used a cocktail of four separate probes in order to increase sensitivity. The importance of this study is that it demonstrates that IL-1 is increased *in vivo* while many previous studies have relied on measuring IL-1 synthesis by glomeruli *in vitro* which may not necessarily reflect in-vivo synthesis.

The signal we have found for IL-1β is clearly localized to nephritic glomeruli and in some glomeruli was clearly localized to individual cells. We have not as yet attempted double labelling to identify the specific cell types synthesizing IL-1. Although IL-1β was originally described as a product of mononuclear phagocytes it is clear that it can also be synthesized by glomerular mesangial cells (Lovett *et al.* 1985; Werber *et al.* 1987). In rabbit anti-gbm glomerulonephritis (Tipping *et al.* 1991) IL-1 production is dependent on macrophage infiltration and macrophages isolated from nephritic glomeruli can produce more than enough IL-1 to account for glomerular production suggesting that macrophages are the predominant source of IL-1. Further evidence for glomerular macrophages as the main source of IL-1 is provided by a study of aminonucleoside nephrosis with double labelling immunohistochemistry (Diamond & Pesek 1991). In our study the peak of IL-1 production at day 2 corresponds with a major increase in macrophage infiltration into the glomerulus which was seen by light microscopy between days 1 and 2.

The role of IL-1 in glomerulonephritis is speculative. Lan *et al.* (1993) have recently demonstrated in accelerated nephrotoxic nephritis that blocking the action of IL-1 with an IL-1 receptor antagonist reduces proteinuria, improves renal function, reduces glomerular hypercellularity and crescent formation and abrogates tubular atrophy and fibrosis. The reduction of glomerular hypercellularity after blocking the effect of IL-1 is consistent with the fact that IL-1 stimulates the production of IL-8, a cytokine with neutrophil chemoattractant properties. The results with the receptor antagonist also suggest that IL-1 is involved in mesangial cell proliferation which is consistent with previously reported effects on cultured mesangial cells (Lovett *et al.* 1983).

In conclusion we have demonstrated by in-situ hybridization that glomerular expression of IL-1β mRNA is enhanced in a model of in-situ immune complex glomerulonephritis, further adding to our understanding of the role of this multifunctional cytokine in glomerulonephritis. Refinement of the method with double labelling may eventually allow cellular localization of IL-1β gene expression.

Acknowledgement

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