Cytokine expression and localization during the development of glomerulosclerosis in FGS mice

Sang-Joon Park[1], Sae-Bom Lee[2], Young-Ho Lee[3], Si-Yun Ryu[4], Kyu-Shik Jeong[5], Cha-Soo Lee[1]

[1]College of Veterinary Medicine, Kyungpook National University, Taegu, Korea
[2]Korea Research Institute of Bioscience & Biotechnology, KIST, Taejon, Korea
[3]College of Medicine, Chungnam National University, Taejon, Korea
[4]College of Veterinary Medicine, Chungnam National University, Taejon, Korea
[5]Lab. of Membrane Biochemistry and Biophysics, NIAAA, NIH, Bethesda, USA

Abstract. To elucidate the mechanism of age-related development in FGS/NgaKIST mice with spontaneous glomerulosclerotic lesion, we examined expression and localization of various cytokine mRNA in the kidney in the progression of diseases. This mouse model is the first to develop spontanously occuring glomerosclerotic lesion in the kidney. In this study, we detected the up-regulation of local cytokine genes such as IL-1β, IL-2, IL-6, IL-10, TNF-α, TGF-β, and IFN-γ in the kidneys. In RT-PCR and Southern blot analysis, we detected gradual expressions of cytokine mRNA of IL-1β, IL-2, IL-6, IFN-γ, and TNF-α mRNA during the course of disease. Other cytokines including IL-10 and TGF-β were found to be appeared the slightly expressed level at 3 to 12 weeks before onset of inflammatory lesion but they are highly expressed at the end-stage of the disease accompaning high proteinurea and wasting. In situ RT-PCR, each cytokine mRNA were specifically localized in a variety of cells including mesangial, endothelial, parietal epithelial, tubular epithelial, arterial muscle cell, and infiltrated inflammatory cells. In addition, TNF-α was detected moderately in the visceral and parietal epithelial cell, but weakly in endothelial and mesangial cells, whereas IL-1β and IL-6 were strong in mesangial regions. IL-6 and TNF-α was highly localized in the damaged proximal and collecting tubules. Especially, TGF-β mRNA was highly found in mesangial cells within glomerulus and interstitium during the end-stage of this disease.. These results indicate that proinflammatory cytokines such as IL-1β, IL-2, IL-6, and TNF-α were gradually expressed from the early stage of this disease to the end-stage, and that IL-10 and TGF-β may be important in the accumulation of extracellular matrix(ECM) within glomerulus and periglomerular fibrosis in the progression of this disease as well as tissue destruction in end-stage of this disease.

Key words : Glomerulosclerosis, cytokine, FGS/NgaKIST mice, RT-PCR, in situ RT-PCR

Introduction

Glomerulosclerosis is the characteristic pathological process by which a variety of different kidney diseases progess to end-stage renal disease. Although various pathogenetic

mechanisms have been proposed, including haemodyamic or permselectivity changes, activation of coagulation, glomerular hypertrophy, especially proteinuria[1], the mechanisms underlying the disease have not yet been elucidated. But several histological studies have revealed considerable pathological abnormalities in this disease. One major histological feature in glomerulosclerosis was associated with the accumulation of extracellular matrix(ECM) within glomerulus and periglomerular fibrosis, mild to moderate interstitial fibrosis and interstitial mononuclear cell infiltration, and occasional tubular dilatation. The onset or progession of this pathological state is associated with abnormal production of one or a set of cytokines. Cytokines are known to play a key role in immune response. Cytokines act in both paracrine and autocrine fashion and are therefore able to induce a medley of reactions involving not only the initiatory cells but also its neighbours. It has become evident that parenchymal kidney cells are able to produce a variety of mediators, including cytokines.[2-5] Cytokines are also produced by infiltration cells, including monocytes and lymphcytes, which are routinely found in the peri-artery and interstitium of damaged kidneys[6,7] and whose presence has prognostic implications.[8]

The pathophysiological responses associated with inflammatory cytokines in renal diseases are currently being characterized. For example, inflammatory cytokines exacerbate many types of glomerulonephritis including mesangial IgA disease, autoimmune lupus nephritis and rejection of renal transplants.[9-12] Although considerable information is available on the types and kinetics of cytokine response in animal models of renal diseases, most of these studies have examined responses in plasma or in isolated glomeruli, mesangial or renal epithelial cells.

In the present study, to determine the roles of cytokines during progressive glomerulosclerosis, we examined various cytokines mRNA expression using RT-PCR method and certified this expression by southern blot analysis in the kidney of a newly established FGS/NgaKIST mouse strain. We also firstly reported the application of in situ RT-PCR to detect the localization of various cytokine mRNA in the kidney of this mouse model

Materials and Methods

Animal

FGS/NgaKist mice were maintained in specific pathogen-free state at KRIBB(korea Research Institute of Bioscience & Biotechnology, KIST, Korea) by brother-sister mating for establishing the inbred strain to F26 generation from F18 generation strain derived from Laboratory of Animal Genetics (School of Agriculture, Nagoya University, Japan). Twenty mice from each FGS/NgaKist mice having the time intervals ranging from 3, 6, 9 12, 15, 18, to 20 weeks and twenty Balb/c mice were used throughout the whole experiment and were maintained on a 12 hr light/dark cycle with autoclaved basal diet and water *ad libitum*. The concentration of urine protein of FGS/NgaKist mice was determined using uropaper (Eiken chemical co., LTD).

Histopathology

The Kidneys were quickly removed and fixed with 10% neutralized buffer formaline(pH 7.2) and cut into 3μm slices for histologic examination. The sections were stained with hematoxylineosin (H-E).

RT-PCR and Southern Blot Analysis

To analyze cytokine gene expression by the RT-PCR, total RNA was extracted from kidney using the Trizol reagent (Gibco BRL) according to the manufacture's protocol.

Briefly, the whole kidney was homogenized in 4 ml of Trizol reagent and precipitated with an equal volume of isopropanol and kept at 20℃ for 2hr. The sample was centrifuged at 12,000X g for 15min to obtain the RNA pellet, which was washed with cold 70 % (v/v) ethanol 3 times for consistency, dried under vacuum and resuspended with diethylpyrocarbonate-treated H^{2O}. RNA yield and purity was determined by measuring optical density at 280 nm and 260 nm. For analysis of cytokine gene expression by RT-PCR, RNA was reverse-transcribed into cDNA. The reaction mixture was added to the RNA solution and incubated at 42℃ for 1hr. Then it was heated at 94℃ for 5min and chilled on ice. For PCR, One-tenth of the cDNA reaction mixture was used and mixed with a 50pmol concentration of the 5′ and 3′ primer, 2.5mM dNTP, 2.5mM MgCl2, and 2U of Taq polymerase (promega). Evaporation was prevented by the addition of 50㎕ of mineral oil and the reaction was carried out by denaturing the RNA-cDNA hybrid by heating at 94℃ for 5mins, annealing the primer at 60℃ for 1min, and extending the primers at 72℃ for 1min. This cycle repeated 30 times in a DNA thermal cycler. After the final cycle, the temperature was maintained at 72℃ for 9mins to allow reannealing of the amplified products, and mixture was then chilled. A 10㎕ aliquot of the amplified DNA reaction mixture was subjected to 1% agarose gel electrophoresis, and the amplified product was visulalized by UV fluorescence. PCR products were blotted onto nylone membrane and hybridized with 32P-labeled specific oligionucleotide probes. Specific cytokine and β-actin primers were designed from Gene Bank data using a computer program and synthesized by using a DNA synthesizer (Bioneer, LTD., Korea). Specific primers used were shown in Table. 1.

Table. 1. Primers for PCR Amplification of Cytokines

Cytokine		Sequence	Size
IL-β	5′	TTGACGGACCCCAAAGAGTG	443bp
	3′	ACTCCTGTACTCGTGGAAGA	
IL-2	5′	CTTGCCCAAGCAGGCCACAG	306bp
	3′	CGAATGTTGTGTATTCCGAG	
IL-4	5′	GAATGTACCAGGAGCCATATC	385bp
	3′	ACCTAATGAGCATCATGACTC	
IL-5	5′	GACAAGCAATGAGACGATGA	235bp
	3′	ACCTAATCCACGTTCTCAAG	
IL-6	5′	CTGGTGACAACCACGGCCTTCCCTA	600bp
	3′	TTGGATCACGCAATACGGATTCGTA	
IL-10	5′	ATGCAGGACTTTAAGGGTTACTTG	254bp
	3′	ATTCGAGGTTCTGGTTCCACAGAT	
TNF-α	5′	GAATGGGTGTTCATCCATTCT	264bp
	3′	GCTTAAGTGACCTCGGAGCTTACA	
TGF-β	5′	AGGAGACGGAATACAGGGCTTTCG	456bp
	3′	CTTCCTGGACCCAACCTTCACCTA	
IFN-γ	5′	AGCGGCTGACTGACTGAACTCAGATTGTAG	306bp
	3′	GGGATATGTCGACTTTTGACACTG	
β-action	5′	ATGGTGGGAATGGGTCAGAAG	169bp
	3′	GGAAGATGTTACTCGACGAGC	

In situ RT-PCR

-pretreatment of specimens

The techniques used were recently been described to detect specific target mRNA within tissues[13], with modifications as described below. Tissue sections were permeabilized with 2mg/ml pepsinogens (Sigma, st Louis, M.O, U.S.A) in 0.01N HCl for 15mins at 25℃ and neutralized with 0.1M Tris-HCl(pH 7.5) and 0.1M NaCl. RQ1 RNase-free DNase (10U/sample)(promega, Madison, W.I, U.S.A.) in 100mM Tris-HCl(pH 8.0), and 50mM MgC^{12}, at 37℃ overnight was used to degrade the DNA. Then DNase was inactivated by heating to 90℃ for 10mins

-In situ reverse transcription

Random hexamer was used in the reverse transcriptase reaction. Reverse transcription was carried out in a total volume of 50㎕ per section reaction. The final concentration of the reaction mixture was as follows: 10mM Tris-HCl, 50mM KCl, 1.5mM MgCl2, 25㎕M deoxynucleotides [dATP, dCTP, dGTP, dTTP] , 100mM DTT in total 40㎕ with a random hexamer and 100U RNasin and 200U Moloney-MLV reverse transcriptase(Perkin Elmer, U.S.A). The slides were incubated with the above reaction mixture for 1hr at 45℃ with a random hexamer. The slides were washed successively with xylene and ethanol series(95-100%), and air dried.

-PCR Amplification

PCR amplification was carried out in 50㎕ of 25㎕M of each the nucleotides dATP, dCTP, dGTP, 23,75㎕M dTTP, 25mM of MgCl2, 10mM Tris-HCl, 50mM KCl and 5U/100㎕ Tag DNA polymerase chain reaction and 100pmoles each of the sense and anti-sense primers and 10U/50㎕ Taq DNA polymerase. The slides were denatured at 95℃ for 1.5mins in the thermocycler(Takara, Japan), annealing at 60℃ for 1min, primer extension at 72℃ for 1min except for the last extension which lasted 12mins for 30 cycles. The slides were rinsed with xylene and ethanol, and air dried.

-Labeling PCR

The DNA fragment synthesized during in situ RT-PCR was labeled with digoxin-11-dUTP in 50㎕ of 25㎕M of each of the nucleotides dATP, dCTP, dGTP, 23.75㎕M dTTP, 10mM Tris-HCl, 50mM KCl, 1.5mM MgCl2, and 5U/100㎕ Taq DNA polymerase. The slides were denatured at 95℃ for 1.5min in the thermocycler(TaKara, Japan), annealing at 60℃ for 1min, primer extension at 72℃ for 1min except for the last extension which lasted 12mins for 5cycles. The slides were rinsed sucessively with xylene and ethanol, and the air dried.

-Immunodetection of PCR product

Briefly, the slides were washed in 0.1M Tris, 0.15M NaCl(pH 7.5), with 2% normal goat serum as a blocking solution to remove unincorporated Dig-dUTP at 45℃ and lay anti-digoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim) diluted in 1:500 in PBS and incubated for 30-60min at room temperature. The specimens were washed in 0.1M Tris(pH 7.5), 0.1M NaCl, 2-3times in Corplin Jar and soaked in 0.1M Tris, 0.1M NaCl, 0.05M MgC^{12}(pH 9.5) for 2min. Five hundreds microliters of chromogens(2.3㎕ of nitroblue tetrazolium salt solution:NBT and 1.8 ㎕ of 5-bromo-4-chloro-3 indole phosphate: BCIP)(Boehringer Mannheim) in soaking buffer and incubated at 37℃ for 15mins in a dark room. The reaction was blocked with two washings of TE buffer. Tissue sections were subsequently dehydrated in a graded series of ethanol dilutions and placed in xylene and mounted on a cover slip.

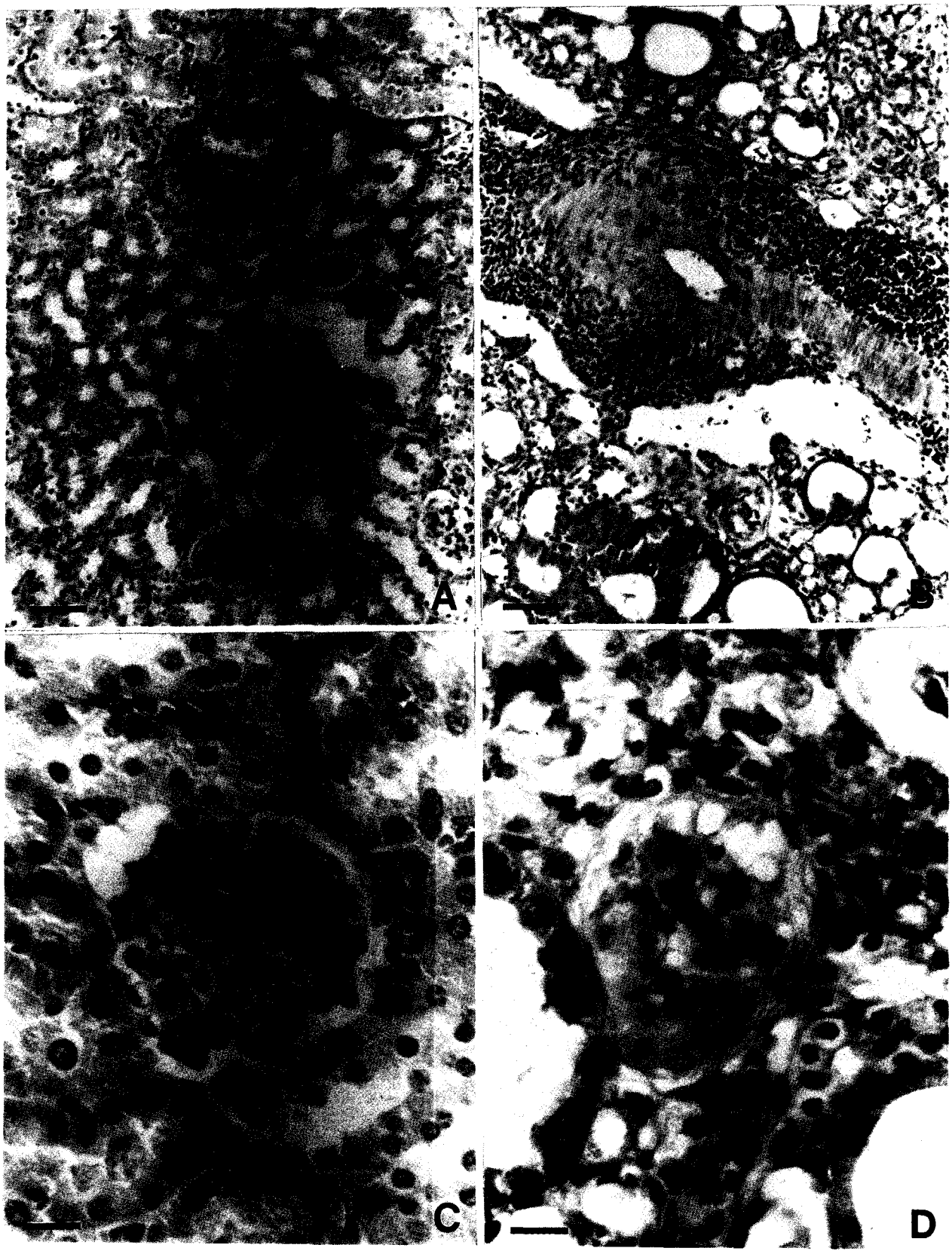

Fig. 1. Kidney; Mice. Histopathology of inflammatory and glomerulosclerotic lesions showing mild(A, C) and severe (B, D) mononuclear cell infiltration in the kidneys of FGS/NgaKist mice from each 15, 20 weeks. A, B; Bar=40 μm. C, D; Bar=20 μm.

Results

Pathological findings

No significant inflammatory lesions and renal changes were found in the kidney of FGS/NgaKist mice until 12 weeks of age before the onset of inflammatory lesion. Most of histological changes appeared to be accompanied with mononuclear cell infiltration around the renal arteries. At 15 weeks, focal glomerulosclerosis and tubular dilatation was scatteringly observed. At 20 weeks, typical histological findings were the accumulation of ECM within glomerulus and periglomerular fibrosis, mild to moderate interstitial fibrosis and mononuclear cell infiltration from periartery to interstitum, and tubular dilatation and atrophy. Figure. 1 shows the representative pathological findings in the kidney with mild(Fig. 1A) and severe inflammatory infiltration(Fig. 1B), and a significant increase of mesangial matrix in the glomeulosclerotic lesions at each 15(Fig. 1C), 20 weeks(Fig. 1D)

Expression of Cytokine Genes

In the kidney of FGS/NgaKist mice from 3 to 20 weeks of age appearing the onset and the progression of inflammatory lesion, we detected gradually expressions of cytokine mRNA of IL-1β, IL-2, IL-6, IFN-γ, and TNF-α mRNA during the course of disease. Other cytokines including, IL-10 and TGF-β were found to be appeared the slightly expressed level at 3 to 12 weeks before onset of inflammatory lesion but they are highly expressed at the end-stage of the disease accompaning high proteinurea and wasting. The most striking expression of these cytokine genes was associated with lymphocytic infiltration and production of high protein urea

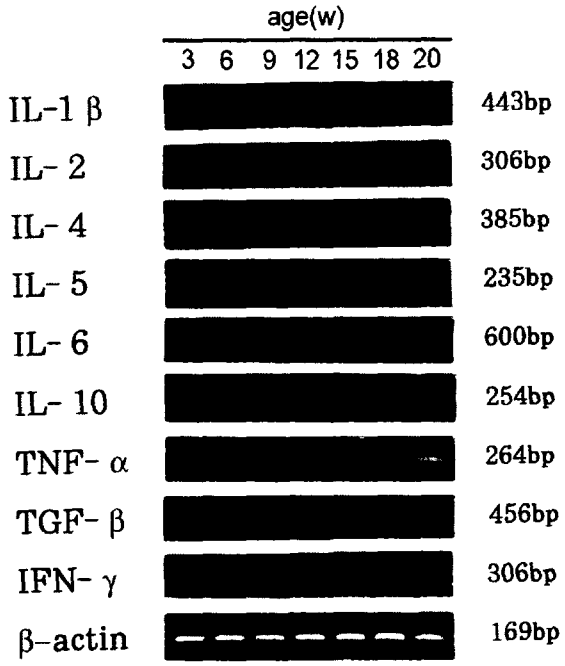

Fig. 2. Expression of cytokines in the kidney of FGS/NgaKist of various ages demonstrated by RT-PCR.

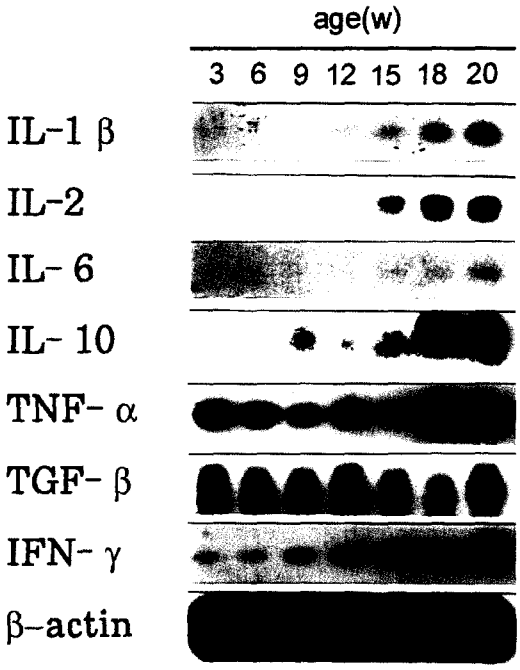

Fig. 3. Expression of cytokines in the kidney of FGS/NgaKist of various ages demonstrated by Southern blot analysis of RT-PCR products.

at the above 15 weeks. But the expression of IL-4 and IL-5 mRNA was not detected throughout the course of disease. Figure. 2, 3 and Table. 2 show the expression of cytokine genes at various ages determined by RT-PCR and Sourthern blot analysis.

In situ RT-PCR

This step was carried out to identify the particular cells responsible for mRNA localization of various cytokines such as IL-1 β, IL-2, IL-6, IL-10, TNF-α, TGF-β, and IFN-γ. A variable proportion of cytokine-posive cells was easily detected in various parenchymal cells containing inflammatory cells in the kidney from the onset of inflammatory lesion at 15 weeks of age, and their numbers increased with age until 20 weeks. Each cytokine mRNA were specifically localized in a variety of cells including mesangial, endothelial, parietal epithelial, tubular epithelial, arterial muscle cell, and infitrated inflammatory cells. Meanwhile, TNF-α was detected moderately in the visceral and parietal epithelial cells(Fig. 4A), but weakly in endothelial and mesangial cells, whereas IL-1β and IL-6 were strong in mesangial cells(Fig. 4B). IL-6 and TNF-α was highly localized in the damaged proximal(Fig. 4C) and collecting tubules(Fig. 4D). Especially, TGF-β mRNA was highly found in mesangial cells(Fig. 4E) within glomerulus and interstitium(Fig. 4F) during the end-stage of this disease. Figure 4 shows the representative findings of in situ RT-PCR in 20 week-old FGS/NgaKist mice. Signal for many cytokines in control mouse was not or weakly found.

Discussion

Although many previous studies of glomerulosclerosis have been attempted investigating the role of many cytokines[2,5,14] little is known in spontaneous anminal model. Many cytokines might be responsible for the developmet of renal disease, but expression level and the acutal localized site of cytokines mRNA has not been analyzed in the progressive glomerular disease. We detected cytokine mRNA levels for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, TGF-β, and IFN-γ in the kidney of FGS/NgaKIST mice at various ages. In this study, expression of IL-1β, IL-2, IL-6, and TNF-α mRNA was gradually increased during the course of disease. Other cytokines including, IL-10 and TGF-β were found to be slightly expressed at 3 to 12 weeks before the onset of inflammatory lesion but they are highly expressed at the end stage of the disease accompaning high proteinurea and wasting. This expression pattern has been showed in the data correlated with IL-1 in the triggering and development of experimentally induced focal glomerulosclerosis[14], and also in the autoimmune MRL-lpr mice model with lupus nephritis appearing enhanced expression of mRNA for TNF-α and IL-1β[15-17]. Although the character of disease differs, these data suggest that cytokines such as TNF-α and IL-1 may be mediators of renal injury. These inflammatory cytokines play a key role as regulatory protein in the progression of glomerulosclerosis and interstitial inflammation during the course of disease. The altered gene expressions of glomerular and non-glomerular cells by genetic and environmental factors trigger increased production and secretion of each cytokines, and of ECM and interstitial fibrotic components.

Recently, there has been great interest in studying the gene expression of various cytokines in renal disease. Most studies have utilized the technique of Northern blotting to demonstrate upregulation of IL-1[15,17,18] and TGF-β[19] mRNA in RNA extracted from either whole kidney or isolated glomeruli in experimental models of glomerulonephritis. In

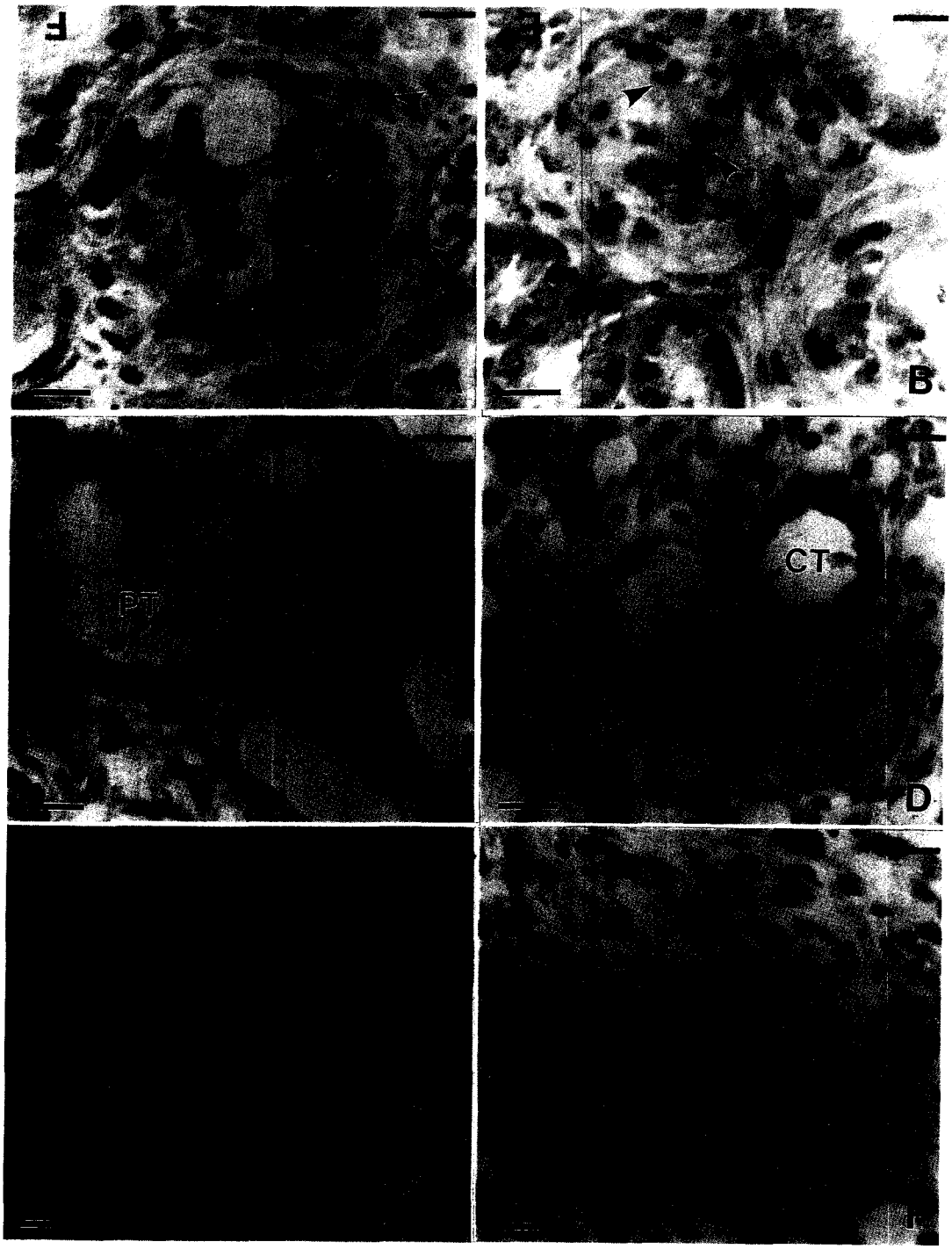

Fig. 4. In situ RT-PCR staining for various cytokines in the kidney of FGS/NgaKist mice with glomerulosclerosis, interstitial fibrosis and tubular dilation at 20weeks. A. TNF-α was detected moderately in the visceral(arrows) and parietal epithelial cell(arrowheads), but weakly in endothelial and mesangial cells, B. IL-1β was strong in mesangial regions(arrows). C, D. IL-6 was highly localized in the damaged proximal tubules(PT)and collecting tubules(CT). E, F. TGF-β mRNA was highly found in mesangial cells within glomerulus(arrowheads) and interstitium. All is Bar=20μm

Table. 2. Kinetic Analysis of Cytokine Gene Expression in the kidney by RT-PCR

Cytokine	FGS/NgaKist mice(week)						
	3 (n=10)	6 (n=10)	9 (n=10)	12 (n=10)	15 (n=5)	18 (n=5)	20 (n=5)
IL-1β	+	++	++	+++	+++	+++	++++
IL-2	±	++	++	++	+++	+++	+++
IL-4	−	−	−	−	−	−	−
IL-5	−	−	−	−	−	−	−
IL-6	+	+	+	±	++	+++	+++
IL-10	+	+	+	+	+	+++	++++
TNF-α	+	+	+	+	+	+++	++++
TGF-β	+	+	+	+	+	+	++++
IFN-γ	+	+	+	+	++	++	++

± ~ ++++ indicate amount of PCR product detectable

this study, the technique of in situ RT-PCR we also used to localize various cytokine mRNA in the kidney of FGS/NgaKIST mice with spontaneous glomerulosclerotic lesion. TNF-α was detected moderately in the visceral and parietal epithelial cell, and weakly in endothelial and mesangial cells, whereas IL-1β and IL-6 were strong in mesangial regions. This findings is in agreement with other report, in which these cytokines may be involved in the mesangial proliferative glomerulonephritis and lupus nephritis. The local increase of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, which initiates immunologic and inflammatory events alone or synergistically, could be associated with persistent tissue damage. An another interesting finding in our study was that kidney tubules express highly IL-6 and TNF-α. Expecially, these cytokines were highly localized in the damaged proximal and collecting tubules, and interstitial cells This finding is in agreement with the previous data that the expression of immunoreactive IL-6 localizes in damaged tubular epithelial cells and various cells[20]. It is believed that IL-6 and TNF-α may act on important factors in the dilation of tubules by the product of oxygen radicals and activation of inflammatory mediator.

On the other hand, TGF-β has lately been focused upon as the major cytokine leading to glomerulosclerosis by the accumulation of ECM and interstitial fibrosis, ultimately leading to the destruction of tissue architecture and thereby to irreversible loss of renal function.[21] In this study, TGF-β was highly expressed and localized in the glomerulus and interstitium during the end stage with renal atropy and elevated proteinuria. This result appear to be key among the mechanisms involved after injury. However, the different pathogenic mechanism between two events such as the loss in the number of glomerular cells and excess interstitial fibrosis may be regulated through different pathway. These events are thought to be related to different cell types and conditions influenced by various stimuli produced through inflammatory response.

In conclusion, we demonstrated that proinflammatory cytokines such as IL-1β, IL-6, and TNF-α gradually influence in the immune

response from early stage of this disease and may have different function in various cells. In addition, IL-10 and TGF-β may be important role in the accumulation of ECM within glomerulus and periglomerular fibrosis in the progression of this disease as well as tissue destruction in end-stage of this disease.

References

1. Klahr S, Schreiner G, et al. The progession of renal disease. N Engl J Med **318**:1657-1666. 1988.
2. Lovett DH and Larsen A. Cell cycle-dependent interleukin 1 gene expression by cultured glomerular mesangial cells. J Immunol 134:1796-1801. 1988
3. Abbound HE, Poptic E, et al. Production of platelet-derived growth factor-like protein by rat mesangial cells in culture. J Clin Invest 80:675-683. 1987
4. Mckay K, Striker LJ, et al. Transforming growth factor-β. J Clin Invest 83:11601167. 1989.
5. Frank J, Engler-Blum G, et al. Human renal tubular cells as a cytokine source:PDGF-B, GM-CSF and IL-6 mRNA expression in vitro. Exp Nephrol 1:26-35. 1993.
6. Hooke DH. Gee DC, et al. Leucocyte analysis using monoclonal antibodies in human glomerulonephritis. Kidney Int 31:964-973. 1987.
7. Markovic-Lipkovski J, Muller CA, et al. Glomerular and interstitial infiltrates of mononuclear leukocytes in glomerulonephritis. Nephrol Dial Transplant 5:10-17. 1990.
8. Bohle A, Mackensen-Haen S, et al. The consequences of tubulo-interstitial changes for renal function in glomerulopathies. A morphometric and cytological analysis. Path Res Prac 186:135-144. 1990.
9. Tam FWK, Karkar AM, et al. Differential expression of macrophage inflammatory protein-2 and monocyte chemoattractant protein-1 in experimental glomerulonephritis. Kidney Int 49:715-721. 1996.
10. Inaba S, Takahashi T, et al. Serum tumor necrosis factor in mesangial IgA glomerulonephritis with macroscopic hematuria in children. Nephron 72:518-522. 1996.
11. Johnson RJ. Cytokine networks and the pathogenesis of glomerulonephritis. J Lab Clin Med 121:190-192. 1993.
12. Suranyi MG, Guasch A, et al. Elevated levels of tumor necrosis factor-α in the nephrotic syndrome in humans. Am J Kidney Dis 21:251-259. 1993.
13. Sun YQ, McLeod, et al. Mechanically induced periosteal bone formation is paralleled by the upregulation of collagen type one mRNA in osteocytes as measured by in situ reverse transcript-polymerase chain reaction. Calcif Tissue Int 57:456-462. 1995.
14. Koichi M and Robert CA. Glomerular cells and Macrophages in the progression of experimental focal and segmental glomerulosclerosis. Am J Pathol 134:933945. 1989.
15. Boswell JM, Yui MA, et al. Increased tumor necrosis factor and IL-β gene expression in the kidneys of mice with lupus nephritis. J Immunol 141:3050-3057. 1988.
16. Boswell JM, Yui MA, et al. Novel and enhanced IL-1 gene expression in autoimmune mice with lupus. J Immunol 141:118-125. 1988
17. Daniel CB, Yui MA, et al. Tumor necrosis factor and IL-1 in new zealand black/white mice enhanced gene expression and acceleration of renal injury. J Immunol 143:3470-3475. 1989.
18. Werber HI, Emancipator SN, et al. The interleukin 1 gene is expressed by rat glomerular

mesangial cells and is augmented in immune complex glomerulonephritis. J Immunol 138:32073212. 1987.

19. Okuda S, Languino LR, et al. Elevated expression of transforming growth factor-β andproteoglycan production in experimental glomerulonephritis. J Clin Invest 86:453462. 1990.
20. Fukatsu A, Matsuo S, et al. Distribution of interleukin-6 in normal and diseased human kidney. Lab Invest 65:61-66. 1991.
21. Border W. Transforming growth factor-β and the the pathogenesis of glomerular disease. Curr Opin Nephrol Hypertens. 3:54-58. 1994.

Request reprints from Dr. Cha-Soo Lee, College of Veterinary Medicine, Kyungpook National University, Taegu, Republic of Korea, 702-701. Tel. & Fax. 053) 950-5966. E-mail: cslee@kyungpook.ac.kr