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Cell-type-specific activation of mitogen-activated protein kinases in PAN-induced progressive renal disease in rats

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Abstract

We examined the time-course activation and the cell-type specific role of MAP kinases in puromycin aminonucleoside (PAN)-induced renal disease. The maximal activation of c-Jun-NH_2-terminal kinase (JNK), extracellular signal regulated kinase (ERK), and p38 MAP kinase was detected on Days 52, 38, and 38 after PAN-treatment, respectively. p-JNK was localized in mesangial and proximal tubular cells at the early renal injury. It was expressed, therefore, in the inflammatory cells of tubulointerstitial lesions. While, p-ERK was markedly increased in the glomerular regions and macrophages p-p38 was observed in glomerular endothelial cells, tubular cells, and some inflammatory cells. The results show that the activation of MAP kinases in the early renal injury by PAN-treatment involves cellular changes such as cell proliferation or apoptosis in renal native cells. The activation of MAP kinases in infiltrated inflammatory cells and fibrotic cells plays an important role in destructive events such as glomerulosclerosis and tubulointerstitial fibrosis.

Keywords: MAP kinase; c-Jun-NH_2-terminal kinase; Extracellular signal regulated kinase; p38 MAP kinase; Progressive renal disease; Inflammation; Glomerulosclerosis; Tubulointerstitial fibrosis

Most progressive renal diseases are characterized by various destructive events during the progression of renal scarring such as glomerulosclerosis and tubulointerstitial fibrosis, which are independent of the initial injury [1]. These pathological processes are largely cellular changes related to the activation of renal parenchymal and infiltrated inflammatory cells through the production of important cellular mediators including cytokines, growth factors, and chemokines. These mediators may lead to proliferative, chemotactic, and particularly destructive events [2,3]. In particular, various stimuli cause activation of signal transduction networks which regulate important cellular changes, including cell proliferation, growth arrest, cell hypertrophy, cell differentiation, and apoptosis. Also, these cellular responses have been demonstrated in a variety of human and experimental renal disease models [4–6].

Mitogen-activated protein (MAP) kinase cascades are among the most thoroughly studied signal transduction pathways. They have been shown to participate in a diverse array of cellular events, transmitting signals from extracellular stimuli such as growth factors, cytokines, and environmental stress in order to activate transcription factors which result in the regulation of gene expression [7]. The three well-characterized kinases are extracellular signal-regulated kinase (ERK), c-Jun NH_2 terminal kinase (JNK), and p38. While ERK responds vigorously to growth factors and certain hormones, JNK and p38 are activated by stress stimuli instead [8]. The activation of these kinases requires phosphorylation of both tyrosine and threonine residues by upstream dual specific kinases. Active MAP kinases are responsible for the phosphorylation of a variety of effector proteins including several nuclear transcription

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factors, such as c-Jun, Elk-1, activated transcription factor (ATF)-2, and c-Fos [9,10].

Recently, the specific signaling pathways for effectors responsible for the progression of renal diseases have been studied extensively through in vivo and in vitro culture systems. Among them, the evidence for the involvement of MAP kinases has been suggested to be activated by numerous mitogens implicated in the pathogenesis of various renal injuries, such as AngII [11], endothelin [12], PDGF [13], cytokines [14], and cellular stress [8,15]. Also, several reports regarding in vivo effect have shown that members of the MAP kinase family are activated in the kidney by specific tissue injuries such as ischemia and reperfusion [16,17], experimental proliferative glomerulonephritis [18], and a diabetic model in rats [6]. Although there are a few in vivo experimental data, many results have mainly focused on the glomerular region through glomeruli isolation or mesangial and tubular cell primary culture. Their regulations and roles in progressive renal disease in vivo, however, remain to be established.

In the present study, special attention has been focused on the role of MAP kinases in both renal native cells and infiltrated inflammatory cells, such as macrophages, lymphocytes, and fibroblast showing a key role in the progression of renal disease. Therefore, we conducted cellular localization using three phosphorylated MAP kinase antibodies to elucidate cell-type specific expression patterns of MAP kinases on whole kidney sections during the development of PAN-induced progressive renal injury in rats. This study is an in vivo demonstration of renal cell-type specific and time-course activation of MAP kinase cascades during the development of PAN-induced progressive renal injury in uninephrectomized rats.

Materials and methods

Experiment design. One hundred female Sprague–Dawley rats weighing 200 g were maintained in a 12 h light–dark cycle with an autocleaved basal diet and water ad libitum. All rats, except for the control rats, had a right unilateral nephrectomy at Day 10 before starting the experiment. This experimental model has been previously reported by Saito et al. [19]. In brief, 42 experimental rats were repeatedly given 1 mg per 100 g body wt over four consecutive days of s.c. PAN at Days 0 and 10, followed by the reduced dosage of 0.5 mg per 100 g of body wt. of s.c. at Days 24 and 38. Control rats received s.c. saline at the same time intervals. All groups of rats were sacrificed on Days 10, 24, 38, 52, 66, and 80.

Histopathology and immunohistochemistry. Rats were anesthetized with an overdose of sodium pentobarbital (100 mg/kg i.p.) and then perfused transcardially with 0.9% saline solution, followed by 4% paraformaldehyde in a 0.1 M phosphate-buffered saline (PBS, pH 7.4). Staining procedures were performed by standard techniques in our laboratory. For immunohistochemistry, the kidneys perfused with 0.9% saline solution and 4% paraformaldehyde in a 0.1 M phosphate-buffered saline (PBS, pH 7.4) were embedded in paraffin. Kidneys were cut with a microtome into coronal sections 3–4 μm thick in a paraffin section. Sections were deparaffinized by immersion in xylene, rehydrated through graded ethanol solutions, and washed in DW. Endogenous peroxidase activity was inhibited using 0.3% hydrogen peroxide in methyl alcohol for 50 min. Sections were then blocked with normal goat serum (Life Technologies, Gaithersburg, MD, USA) for 60 min and incubated overnight at 4 °C with anti-phospho-JNK (1:200, Santa Cruz Biotechnology, CA, USA), anti-phospho-ERK (1:200, Santa Cruz Biotechnology, CA, USA) or anti-phospho-p38 (1:200, Santa Cruz Biotechnology, CA, USA) antibodies diluted in a PBS (pH 7.4). After washing with a PBS (pH 7.4), slides were incubated with a biotin-conjugated secondary antibody (Vector Laboratoris, Burlingame, CA) and diluted in a PBS (pH 7.4) for 1 h at room temperature. Sections were rinsed with a PBS (pH 7.4) for 10 min three times and then incubated with an avidin–biotin reagent (Vector Laboratoris, Burlingame, CA) for 1 h at room temperature. Sections were washed with a PBS (pH 7.4) and color was developed using a commercial 3,3′-diaminobenzidine (Zymed, San Francisco, CA, USA) as the chromogen. Tissue sections were then rinsed in distilled water and counterstained in Mayer's hematoxylin. The control included sections stained after the omission of the primary antibody. In addition, specific antibodies for $CD4^+$ (W3/25) and $CD8^+$ T cells (OX-8), macrophages (ED-1), and myofibroblasts (α-smooth muscle actin) were used to characterize the cells related with the activation of JNK, ERK, and p38 MAP kinase in serial sections.

Immunoprecipitation and immunoblot analysis. Whole cortical tissues were homogenized by a polytron homogenizer on ice in a lysis buffer containing 25 mM Hepes (pH 7.5), 300 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM DTT, 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 2 μg/ml leupeptin, and 100 μg/ml PMSF. Homogenates were centrifuged at 15,000 rpm for 20 min at 4 °C and the protein content of lysates was determined using the Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA, USA). Proteins (500 μg/sample) extracted from whole cortical kidney tissue were incubated with 2 μg of antibodies for JNK1/2, ERK2, and p38 at 4 °C for 2 h with rotation. Protein A/G-agarose beads (40 μl/sample) were then added to each sample and incubated for an additional 2 h at 4 °C with rotation. The immune complexes precipitated with protein A/G-agarose were washed three times with a buffer containing initial tissue homogenized buffer and then were mixed 5× sample buffer and heated for 5 min at 95 °C. Denatured protein lysates were loaded per lane and separated by SDS–PAGE using 4% and 10% acrylamide for stacking and resolving gels, respectively. Protein was transferred to a nitrocellulose membrane and probed with monoclonal antibodies against phosphorylated or non-phosphorylated JNK, ERK and p38 (Santa Cruz Biotechnology, CA, USA). The primary antibodies (diluted 1:1000) were detected using horseradish peroxidase (HRP)-conjugated anti-mouse or rabbit IgG (Calbiochem, USA) and visualized by an Amersham ECL system (Braunschweig, Germany) after extensive washing of the membranes.

Quantification of morphological data. All quantification was performed in a blind manner. The quantitation of immunohistochemical staining for each MAP kinase on tissue was performed on 40 randomly selected glomeruli and 100 tubular regions of the cortical area under 400× magnification in each sample of the seven animals.

Statistical analysis. Densitometry data from the immunoblot were subjected to Student's *t* test to determine the difference of protein levels between the samples. Results were considered to be significant if P was <0.05 or 0.001. Data are presented as means with SE.

Results

JNK activation in PAN-induced renal injury

To determine whether MAP kinases are involved in the progression of PAN-induced renal injury, we examined the phosphorylated form of each MAP kinase.

Phosphorylated JNK increased at Days 10 and 38, was maximal at Day 52, and sustained at Day 66; therefore, they returned to near basal levels at Day 80 (Fig. 1A, upper photograph). Fig. 1A showed that there weren't any changes found in the total level of JNK protein at each time-course progression. In the immunohistochemistry for specific phosphorylated JNK, this kinase was localized in mesangial and endothelial cells of glomeruli as well as dilated tubular cells at Day 10 after PAN treatment (Fig. 2B). Thereafter, phosphorylated JNK positive cells were present in periglomerular and tubulointerstitial lesions as well as in various arteries. In particular, specific phosphorylated JNK was expressed in maximum levels in various infiltrated cells and fibroblasts on Days 38 and 52 (Figs. 2C and D and 4A, right bar-graph). On Day 80, although infiltrated lymphatic and fibrotic cells increased in the interstitium, the expression for phosphorylated JNK decreased in the renal scarring lesions showing entirely tubulointerstitial fibrosis. It is interesting to note that infiltrated inflammatory cells showing phosphorylated JNK positive staining during the progression of disease were further identified with most of the cytotoxic $CD8^+$ T cells (Figs. 3A and B) and some macrophages. Also, immunohistochemical positive cells for α-SMA were mainly localized in phosphorylated JNK positive regions such as periglomerulus with sclerotic changes and peritubular lesions showing severe dilation and atrophy (Figs. 3C and D). Also, these cell types increased gradually during the development of the disease. Fig. 4A shows the time-course expression of phosphorylated JNK in the glomeruli and tubulointerstitial regions of PAN-induced rats in the seven animals of each group.

ERK activation in PAN-induced renal injury

An increase of phosphorylated ERK kinase was found between Days 10 and 24, was at a maximum level at Day 38, and sustained between Days 52 and 66. Thereafter, they returned to near basal levels at Day 80 (Fig. 1B, upper photograph). Fig. 1B showed that no changes were found in the total level of ERK protein at each time-course progression. The immunohistochemical detection of specific phosphorylated ERK revealed intense immunoreactivity in the mesangial region on Day 24 (Fig. 2F) and infiltrated macrophages on Day 38 (Fig. 2G) were identified with a positive cell for ED-1. Macrophages with positive immunoreactivity for ED-1 were localized in the glomerular and tubulointerstitial lesions during the course of the disease (Fig. 3). Fig. 4B shows the time-course comparison of phosphorylated ERK in the glomeruli and tubulointerstitial regions of PAN-induced rats in the seven animals of each group.

P38 activation in PAN-induced renal injury

Immunoblot analysis for phosphorylated p38 kinase in PAN-induced renal injury showed that this kinase started to increase at Day 10, was maximal at Day 52, and sustained during the whole experiment (Fig. 1C, upper photograph). Fig. 1C showed that no changes were found in the total level of p38 protein at each time-course progression. In immunohistochemistry, phosphorylated p38 kinase was observed in the renal tubular cells and glomerular endothelial cells showing moderate to intense immunoreactivity at 10 day (Fig. 2J) and 38 day (Fig. 2K). As the disease progressed, this kinase was also localized in various infiltrated inflammatory cells. Fig. 4C shows the time-course comparison of phosphorylated p38 kinase in the glomeruli and tubulointerstitial regions of PAN-induced rats in the seven animals of each group.

Discussion

MAP kinases play a pivotal role in the regulation of important cellular functions by the activation of specific

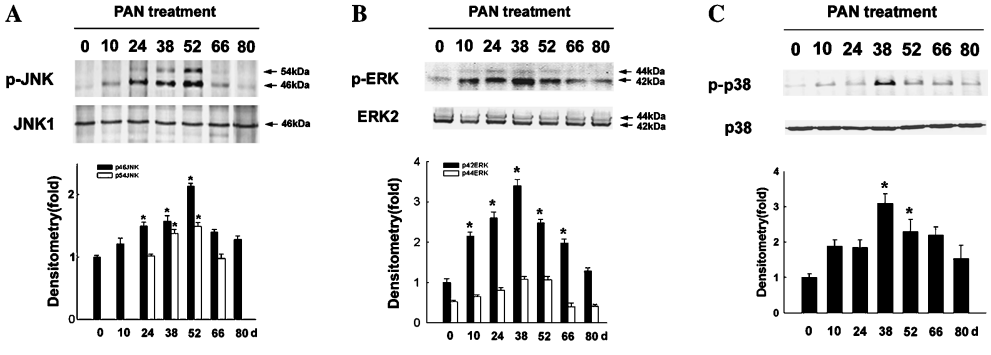

Fig. 1. JNK, ERK, and p38 activation in the whole cortical tissue of rat kidneys in the PAN-induced progressive renal disease. Rats were sacrificed at indicated time points after the PAN treatment, and whole cortical tissues were homogenized. Total JNK, ERK, and p38 antibodies (1 mg or 400 μg in 0.5 ml lysis buffer) were immunoprecipitated from the protein extracts and their kinase activities were measured based on the phosphorylated state of each JNK, ERK, and p38 (upper photograph), respectively. The bars of the total JNK, ERK, and p38 show equal amounts of each protein. The bar graphs show the representative density of phosphorylated JNK, ERK isoforms, and p38 at each time point, respectively. Each value represents mean ± SD, $n = 7$. $^*P < 0.01$ compared with the control group.

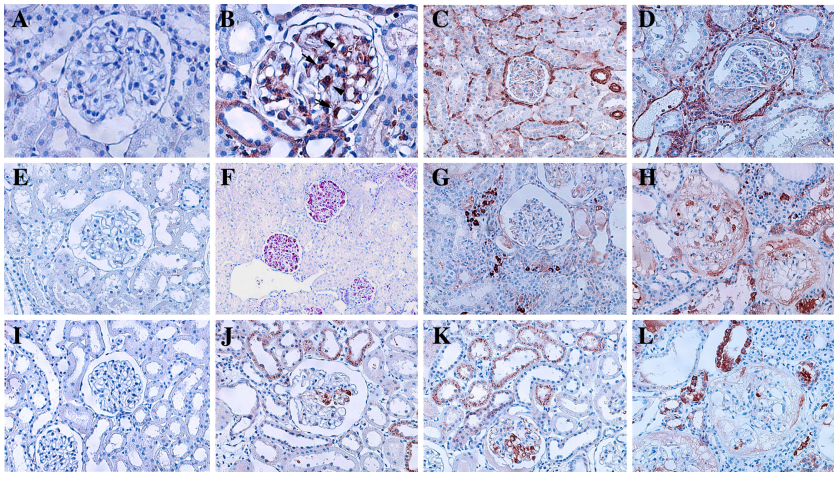

Fig. 2. Immunohistochemical findings for phosphorylated JNK, ERK, and p38 of rat kidneys in PAN-induced progressive renal disease. (A) Immunohistochemical findings for p-JNK of control rats (400×). (B) p-JNK positive cells localized in mesangial (arrowheads) and endothelial (arrows) cells at Day 10 after PAN treatment (400×). (C,D) Increased p-JNK positive cells in periglomerular and peritubular regions at Days 38 and 52 after PAN treatment, respectively (200×). (E) Immunohistochemical findings for p-ERK of control rats (200×). (F) p-ERK positive cells localized in the glomerular regions showing proliferation at Day 10 after PAN treatment (100×). (G) p-ERK positive cells localized in infiltrated inflammatory cells at Day 38 after PAN treatment (200×). (H) p-ERK positive cells decreased in the glomerular and tubular regions at Day 66. (I) Immunohistochemical findings for p-p38 of control rats (200×). (J) p-p38 positive cells localized in the glomerular and tubular cells showing abnormal histological findings at Day 24 after PAN treatment (200×). (K) p-p38 positive cells localized in damaged glomerular endothelial and tubular cells at Day 38 after PAN treatment (200×). (L) Some p-p38 positive cells localized in the glomerular region and tubular cells at Day 80 after PAN treatment (200×).

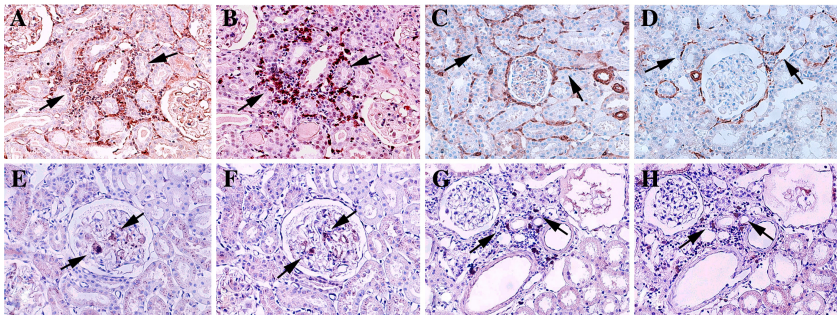

Fig. 3. Colocalization of phosphorylated JNK and ERK using cell type specific markers in rat kidneys of a PAN-induced progressive renal disease. To characterize cells expressing p-JNK and p-ERK, tissue sections were stained with specific antibodies for $CD8^+$ T cells (OX-8), myofibroblasts (α-SMA), macrophages (ED-1). (A) p-JNK positive cells localized in inflammatory and fibrotic cells (arrows) at Day 52 in a serial section with (B) (200×). (B) $CD8^+$ T cells localized in infiltrated inflammatory cells (arrows) at 52 day and when compared with (A) serial-sectioned, it shows the colocalization of $CD8^+$ T cells and p-JNK positive cells in infiltrated inflammatory cells at Day 52 (200×). (C) p-JNK positive cells localized in fibrotic cells (arrows) at Day 52 in compared with (D) (200×). (D) α-SMA positive cells localized in tubulointerstitial regions (arrows) at Day 52 (200×). (E,F) Colocalization for p-ERK (E, arrows) and ED-1 positive cells (F, arrows) in serial sections of glomerular regions at Day 24 after the PAN treatment, respectively (200×). (G,H) Colocalization for p-ERK (G) and ED-1 positive cells (H) in the serial sections of tubulointerstitial regions at Day 38 after the PAN treatment, respectively (200×).

signal transduction pathways from the cell surface to the nuclei. The JNK, ERK, and p38 enzymes are the terminal enzymes of the three subfamilies in the MAP kinases, each of which consists of a cascade of kinases, where each kinase phosphorylates and thereby activates the next family in the sequence [7,15]. It has been suggested that these kinases are activated by numerous factors implicated in the pathogenesis of various renal injuries, including AngII [11], endothelin [12], PDGF [13], cytokines [14], and cellular stress [8]. The resolutions of these implicated pathomechanisms have been studied by direct injury effect through in vitro studies using cultured mesangial cells, glomerular epithelial cells, endothelial cells, and tubular cells. Their regulations and roles in progressive renal diseases in vivo, however, remain unknown.

Recently, in vivo studies also suggest that members of the MAP kinase family are activated in the kidney by specific tissue injuries such as ischemia and reperfusion [16,17], experimental proliferative glomerulonephritis [18], and a diabetic model [6] in rats. Although there are little in vivo experimental data, most results are

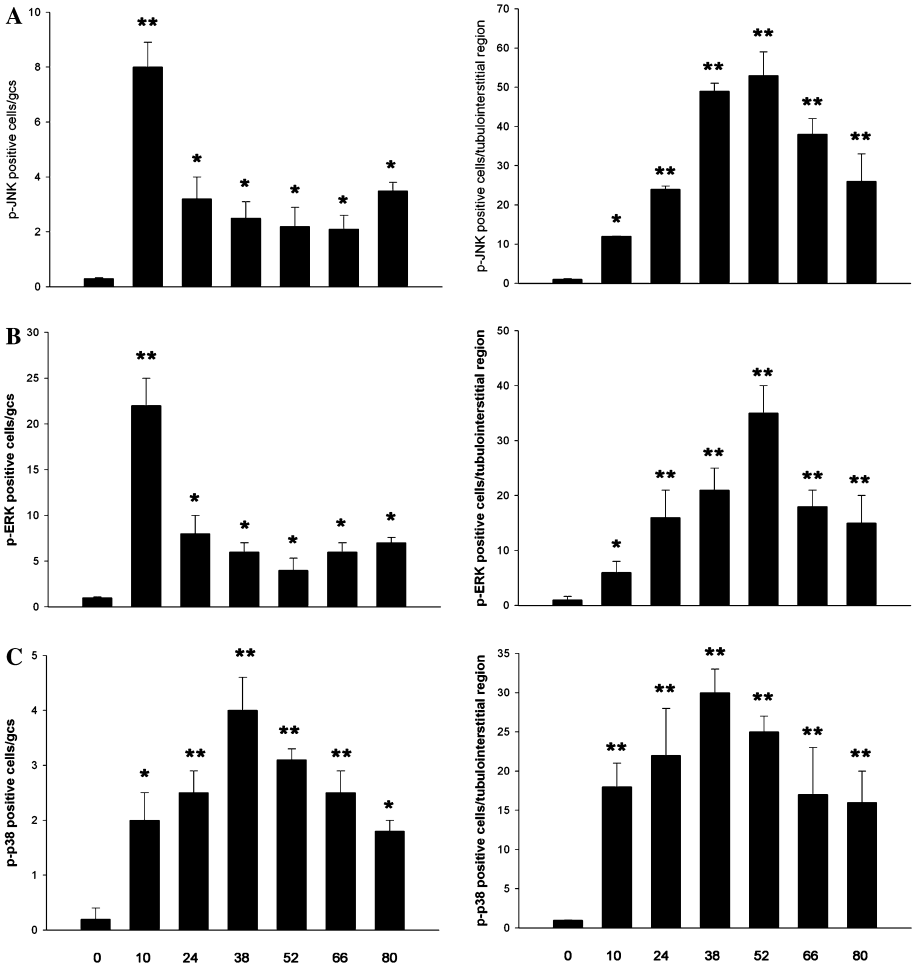

Fig. 4. Quantification of immunohistochemistry for p-JNK, p-ERK, and p-p38 in the glomeruli and tubulointerstitial regions of rat kidneys in PAN-induced progressive renal disease. The number of p-JNK, p-ERK, and p-p38 positive cells was counted in the glomeruli and tubular regions of normal groups and PAN-treated groups. The data are represented as means ± SD number of positive cells per 40 randomly selected glomeruli and the 100 tubular regions of the cortical area, under 400× magnification in the seven animals of each group. *$P < 0.05$; **$P < 0.001$ compared with normal animals.

mainly focused on the glomerular region through glomeruli isolation or mesangial cell primary culture. In the present study, we provide the first in vivo demonstration of renal cell-type specific and the disease time-course activation of MAP kinase cascade during the development of PAN-induced progressive renal injury in uninephrectomized rats. It is generally observed that the early activation of MAP kinase cascades in many in vivo models was reported to be regulated by the initial cellular responses through cell proliferation or differentiation by cytokines and growth factors in the cellular processes such as hypertrophy and hyperplasia in glomeruli of rat models of glomerulonephritis and diabetic nephropathy [6,20]. In this study, the initial increase of phosphorylated ERK levels was also observed between Days 10 and 24 in accordance with renal hypertrophy showing the increase of kidney sizes in this model. These sizes reached a maximum level at Day 38 and were sustained between Days 52 and 66. Thereafter, they returned to near basal levels at Day 80 in the time-course expression pattern of this kinase during the progression of the PAN-induced renal injury. The markedly increased and sustained phosphorylation of ERK in accordance with the pathological findings suggests chronic activation of the ERK through various cellular changes. A similar ERK activity was demonstrated in the glomeruli of anti-GBM or anti-Thy-1 glomerular nephritis model in rats [18]. In this study, the distribution of activated ERK was verified by immunohistochemistry using phosphorylated ERK antibody. In immunohistochemistry, we demonstrated that the mesangial cells and proximal tubular cells as well as infiltrated macrophages within the glomeruli are major cell components of ERK activation through the cellular localization of phosphorylated ERK. Recently, Bokemeyer et al. [18] reported that ERK kinase is activated significantly in the cortical tissue of kidneys showing proliferative glomerulonephritis in response to immune injury. In addition, ERK

activities have been proposed that resident glomerular cells rather than the infiltrating cells are the site with altered intracellular MAP kinase signaling because of the kinetics of the infiltrated inflammatory cells such as initial increase and later decrease in the number of these infiltrating cells. Our results suggest that it is important not only in the cellular changes of glomerular cells but also in the activation of infiltrated macrophages.

Unlike ERK, JNK is generally suggested to inhibit cellular proliferation and to induce apoptosis [21,22]. In addition, this kinase has been shown to play a critical role in the events leading to activation in inflammatory cells [23]. In the present study, we examined the phosphorylated JNK to determine whether it is involved in the activation of cellular signaling during the progression of a PAN-induced renal injury. The activation of phosphorylated JNK increased at Days 10 and 38 was maximal at Day 52 and sustained at Day 66; thereafter, they returned to near basal levels at Day 80. In the immunohistochemistry for specific phosphorylated JNK antibody, this kinase was mainly localized in the mesangial and endothelial cells, as well as in damaged tubular cells at Day 10 after the initial PAN treatment. At this point in time, the localization of these cells is regarded as the cellular response which is regulated through direct effect and toxic proteinuria by PAN treatment. In addition, this kinase was localized in small numbers of infiltrated lymphatic cells and fibroblasts at the same time. The number of infiltrated lymphatic cells and fibroblasts gradually increased. The number correlates closely with the severity of the glomerular and tubulointerstitial lesions as well as the loss of renal function during the progression of the diseases. These cells were mainly present in the peri-glomerular and tubulointerstitial lesions as well as various peri-arteries. The localization of these cell types increased gradually until Day 66. In particular, on Day 52, specific phosphorylated JNK was expressed in maximum levels in a variety of infiltrated lymphatic and fibrotic cells. On Day 80, although the number of infiltrated lymphatic and fibrotic cells increased in the glomerular and tubulointerstitial lesions, the expression for phosphorylated JNK decreased in the end stage of the renal scarring process, showing chronic pathological findings such as glomerulosclerosis and tubulointerstitial fibrosis. These findings are explained as being due to the loss of JNK activity in these cell-types. The inflammatory cells that infiltrated the most were identified as cytotoxic T cells by immunohistochemistry using specific inflammatory cell markers for macrophage, $CD4^+$ T cell and $CD8^+$ T cell. Studies in several models of inflammatory renal disease have demonstrated that T cells were an important component of the renal lesion [24–28]. Specifically, $CD8^+$ T cells have been proven to play an important effector role in the various types of immune-mediated renal disease [24]. T cell activation involves several steps including the induction of immediate early genes, followed by the induction of interleukin-2 (IL-2), whose secretion leads to autocrine and paracrine T cell proliferation [28].

Another positive cell type for phosphorylated JNK is the fibrotic cells of various tubular interstitial and glomerular regions, which show the progression of renal scarring such as tubulointerstitial fibrosis or glomerulosclerosis. The characteristics of fibrotic cells in the renal scarring process have been extensively investigated, and most of them have been considered as being transformed into myofibroblasts during the progression of a disease. Myofibroblasts are particularly important, since they are the major producers of extracellular matrix (ECM) proteins [29]. Several cell types present in the kidney are potential candidates as precursors of myofibroblasts, which appear to originate from fibroblasts under the influence of various factors. Among these factors, TGF-β1 is well known to induce the phenotypic modulation of fibroblasts to activated myofibroblasts, and the increased expression of TGF-β1 is associated with active fibrosis, which has been shown to occur in renal scarring [30]. Hashimoto et al. reported that TGF-β1 induces the phenotypic modulation of human lung fibroblasts to myofibroblasts through the JNK-dependent pathway [31,32]. Hocevar et al. [33] demonstrated that TGF-β-mediated fibronectin induction requires the activation of JNK, which in turn, modulates the activity of c-Jun and ATF-2 in a Smad4-independent manner. In the present study, phosphorylated JNK was localized in the fibrotic cells of the tubulointerstitial region, as well as glomerulus showing glomerulosclerosis with the severity of its disease. It is considered that the increase of ECM proteins through the activation of fibrotic cells through the JNK signal pathway plays an important role in tubulointerstitial fibrosis.

Although the roles of the p38 signal pathway, unlike JNK and ERK, are poorly understood in renal disease, there are possibilities for its activation by various renal injury factors and abnormal cellular microenvironment in renal disease. Based on in vitro experiments, the p38 pathway is considered as one of the stress response pathways, and traditionally is thought to play a role in inflammation, immune activation, growth arrest, and apoptosis [34–36]. In the present study, we investigated whether p38 kinase participates in the progression of renal disease in rats after the PAN treatment. The phosphorylated p38 kinase increased along with the time-course dependency of the disease. By immunohistochemistry, phosphorylated p38 was detected in tubular epithelial cells showing abnormal morphology on Days 10 and 24. Although we have not identified the possible factors for the activation of the p38 pathway in PAN-induced renal injury, it may be speculated that the direct toxic effect of PAN or hypoxic state in the tubules induces phosphorylation of p38 kinase. In particular, in accordance with the development of the

disease, this kinase was also localized in multiple infiltrated inflammatory cells such as T cells and macrophages. These results suggest that p38 kinase activation may play an important role in mediating the progression of renal disease through the interplay of inflammatory and residual cells. Furthermore, this kinase may involve the apoptosis of tubular cells and infiltrated inflammatory cells through the activation of the p38 signal pathway.

From these data, the activations of JNK, ERK, and p38 MAP kinase were each found at distinct kidney sites in PAN-induced progressive renal disease, in particular, the early stages of renal disease involved compensatory renal responses, which are associated with cellular hypertrophy, hyperplasia, and apoptosis. As these cellular responses progress as a further chronic renal diseased stage, glomerular and tubulointersitial changes may be regulated through the interactions between residual renal cells and the activated inflammatory cells via ERK, JNK, and p38 signaling pathways. This indicates that the chronic activation of these kinases may play an important role in events related with the increase of extracellular matrix proteins. In conclusion, it might be more of an additional stress if the specific intracellular signaling modules are controlled rather than the blocking of any extracellular targets in preventing various renal diseases.

Acknowledgment

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References

[1] L.N. Irene, K.F. Clarice, Z. Roberto, The inflammatory component in progressive renal disease-are interventions possible? Nephrol. Dial. Transplant. 17 (2002) 363–368.
[2] A.V. Cybulsky, Growth factor pathways in proliferative glomerulonephritis, Curr. Opin. Nephrol. Hypertens 9 (2000) 217–223.
[3] W.W. Tang, M. Qi, J.S. Warren, G.Y. Van, Chemokine expression in experimental tubulointerstitial nephritis, J. Immunol. 159 (1997) 870–876.
[4] D. Bokemeyer, K.E. Guglielmi, A. McGinty, A. Sorokin, E.A. Lianos, M.J. Dunn, Activation of extracellular signal-regulated kinase in proliferative glomerulonephritis in rats, J. Clin. Invest. 100 (1997) 582–588.
[5] D. Bokemeyer, D. Panek, H. Kramer, M. Lindemann, M. Kitahara, P. Boor, D. Kerjaschki, J. Trzaskos, J. Floege, T. Ostendorf, In vivo identification of the mitogen-activated protein kinase cascade as a central pathogenic pathway in experimental mesangioproliferative glomerulonephritis, J. Am. Soc. Nephrol. 13 (2002) 1473–1480.
[6] M. Haneda, S. Araki, M. Togawa, T. Sugimoto, M. Isono, R. Kikkawa, Activation of mitogen-activated protein kinase cascade in diabetic glomeruli and mesangial cells cultured under high glucose conditions, Kidney Int. Suppl. 60 (1997) S66–S69.
[7] L. Chang, M. Karin, Mammalian MAP kinase signalling cascades, Nature 410 (2001) 37–40.
[8] M.K. John, A. Joseph, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation, Physiol. Rev. 81 (2001) 807–869.
[9] R. Treisman, Regulation of transcription by MAP kinase cascades, Curr. Opin. Cell Biol. 8 (1996) 205–215.
[10] M. Cavigelli, F. Dolfi, F.X. Claret, M. Karin, Induction of c-fos expression through JNK-mediated TCF/Elk-1 phosphorylation, EMBO J. 14 (1995) 5957–5964.
[11] D.F. Liao, B. Monia, N. Dean, B.C. Berk, Protein kinase C-ζ mediates angiotensin II activation of ERK 1/2 in vascular smooth muscle cells, J. Biol. Chem. 272 (1997) 6146–6150.
[12] Y. Wang, M.S. Simonson, J. Pouyssegur, M.J. Dunn, Endothelin rapidly stimulates mitogen-activated protein kinase activity in rat mesangial cells, Biochem. J. 287 (1992) 589–594.
[13] A. Huwiler, S. Stabel, D. Fabbro, J. Pfeilschifter, Platelet-derived growth factor and angiotensin II stimulate the mitogen-activated protein kinase cascade in renal mesangial cells: comparison of hypertrophic and hyperplastic agonists, Biochem. J. 305 (1995) 777–784.
[14] W.A. Wilmer, L.C. Tan, J.A. Dickerson, M. Danne, B.H. Rovin, Interkeukin-1β induction of mitogen-activated protein kinases in human mesangial cells: role of oxidation, J. Biol. Chem. 272 (1997) 10877–10881.
[15] E. Cano, L.C. Mahandevan, Parallel signal processing among mammalian MAPKs, Trends Biochem. Sci. 20 (1995) 117–122.
[16] H. Morooka, J.V. Bonventre, C.M. Pombo, J.M. Kyriakis, T. Force, Ischemia and reperfusion enhance ATF-2 and c-Jun binding to cAMP response elements and to an AP-1 binding site from the c-Jun promoter, J. Biol. Chem. 270 (1995) 30084–30092.
[17] C.M. Pombo, J.V. Bonventre, J. Avruch, J.R. Woodgett, J.M. Kyriskis, T. Force, The stress-activated protein kinases (SAPKs) are major c-Jun aminoterminal kinases activated by ischemia and reperfusion, J. Biol. Chem. 269 (1997) 26546–26551.
[18] N.W. Boyce, S.R. Holdsworth, C.D. Dijikstra, R.C. Atkins, Activation of extracellular signal-regulated kinase in proliferative glomerulonephritis in rats, J. Clin. Invest. 100 (1997) 582–588.
[19] T. Saito, E. Sumithram, E.F. Glasgow, R.C. Atkins, The enhancement of aminonucleoside nephrosis by the co-administration of protamine, Kidney Int. 32 (1987) 691–699.
[20] K. Matsumoto, R.C. Atkins, Glomerular cells and macrophages in the progression of experimental focal and segmental glomerulosclerosis, Am. J. Pathol. 134 (1989) 933–945.
[21] Z. Xia, M. Dickens, J. Raingeaud, R.J. Davis, M.E. Greenberg, Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis, Science 270 (1995) 1326–1331.
[22] B.W. Zanke, K. Boudreau, E. Rubie, E. Winnett, L.A. Tibbles, L. Zon, J. Kyriakis, F.F. Liu, J.R. Woodgett, The stress-activated protein kinase pathway mediates cell death following injury induced by *cis*-platinum, UV irradiation or heat, Curr. Biol. 6 (1996) 606–613.
[23] Y.T. Ip, R.J. Davis, Signal transduction by the c-Jun N-terminal kinase (JNK)-from inflammation to development, Curr. Opin. Cell Biol. 10 (1998) 205–219.
[24] Y. Wang, P.W. Wang, Y.C. Tay, C.H. Harris David, Role of CD8 cells in the progression of murine adriamycin nephropathy, Kidney Int. 59 (2001) 941–949.
[25] T. Kuroda, K. Kawasaki, T. Oite, M. Arakawa, F. Shimizu, Nephrotoxic serum nephritis in nude rats—the role of cell-mediated immunity, Nephron 68 (1994) 360–365.
[26] M.J. Penny, R.A. Boyd, B.M. Hall, Permanent $CD8^+$ T cell depletion prevents proteinuria in active Heymann nephritis, J. Exp. Med. 188 (1998) 1775–1784.
[27] C.M. Meyers, C.J. Kelly, Effector mechanisms in organ-specific autoimmunity. I. Characterization of a CD8+ T cell line that

mediates murine interstitial nephritis, J. Clin. Invest. 88 (1991) 408–416.
[28] G.R. Crabtree, Contingent genetic regulatory events in T lymphocyte activation, Science 243 (1989) 355–361.
[29] E.C. Muchaneta-Kubara, A.M. El Nahas, Myofibroblast phenotypes expression in experimental renal scarring, Nephrol. Dial. Transplant. 12 (1997) 904–915.
[30] D.S. Goumenos, A.C. Tsamandas, S. Oldroyd, F. Sotsiou, S. Tsakas, C. Petropoulou, D. Bonikos, A.M. El Nahas, J.G. Vlachojannis, Transforming growth factor-beta(1) and myofibroblasts: a potential pathway towards renal scarring in human glomerular disease, Nephron 87 (2001) 240–248.
[31] S. Hashimoto, Y. Gon, I. Takeshita, K. Matsumoto, S. Maruoka, T. Horie, Transforming growth Factor-beta1 induces phenotypic modulation of human lung fibroblasts to myofibroblast through a c-Jun-NH2-terminal kinase-dependent pathway, Am. J. Respir. Crit. Care. Med. 163 (2001) 152–157.
[32] M. Utsugi, K. Dobashi, T. Ishizuka, K. Masubuchi, Y. Shimizu, T. Nakazawa, M. Mori, C-Jun-NH2-terminal kinase mediates expression of connective tissue growth factor induced by transforming growth factor-beta1 in human lung fibroblasts, Am. J. Respir. Cell Mol. Biol. 28 (2003) 754–761.
[33] B.A. Hocevar, T.L. Brown, P.H. Howe, TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway, EMBO J. 18 (1999) 1345–1356.
[34] C. Stambe, R.C. Atkins, P.A. Hill, D.J. Nikolic-Paterson, Activation and cellular localization of the p38 and JNK MAPK pathways in rat crescentic glomerulonephritis, Kidney Int. 64 (2003) 2121–2132.
[35] B.F. Liu, S. Miyata, Y. Hirota, S. Higo, H. Miyazaki, M. Fukunaga, Y. Hamada, S. Ueyama, O. Muramoto, A. Uriuhara, M. Kasuga, Methylglyoxal induces apoptosis through activation of p38 mitogen-activated protein kinase in rat mesangial cells, Kidney Int. 63 (2003) 947–957.
[36] T. Wada, K. Furuichi, N. Sakai, Y. Hisada, K. Kobayashi, N. Mukaida, N. Tomosugi, K. Matsushima, H. Yokoyama, Involvement of p38 mitogen-activated protein kinase followed by chemokine expression in crescentic glomerulonephritis, Am. J. Kidney Dis. 38 (2001) 1169–1177.