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Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy

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Background. We previously described that monocyte chemoattractant protein-1 (MCP-1) plays an important role in progressive glomerular and interstitial damage in inflammatory renal diseases. However, the expression of MCP-1 in diabetic nephropathy remains to be investigated.

Methods. We examined whether locally expressed MCP-1 participates in human diabetic nephropathy via recruiting and activating monocytes/macrophages (Mϕ). Urinary and serum MCP-1 levels were measured by enzyme-linked immunosorbent assay in 45 patients with diabetic nephropathy. The presence of MCP-1 in diseased kidneys was determined by immunohistochemical and in situ hybridization analyses.

Results. Urinary MCP-1 levels were significantly elevated in patients with diabetic nephrotic syndrome and advanced tubulointerstitial lesions. Moreover, urinary levels of MCP-1 were well correlated with the number of CD68-positive infiltrating cells in the interstitium. In contrast, serum MCP-1 levels remained similar to those of healthy volunteers. Furthermore, we detected the MCP-1–positive cells in the interstitium of diabetic nephropathy via both immunohistochemical and in situ hybridization analyses.

Conclusion. These observations suggest that locally produced MCP-1 may be involved in the development of advanced diabetic nephropathy, especially in the formation of tubulointerstitial lesions possibly through Mϕ recruitment and activation. Moreover, up-regulation of MCP-1 may be a common pathway involved in the progressive tubulointerstitial damage in diabetic nephropathy as well as inflammatory renal diseases.

The accumulation of matrix proteins resulting in glomerular sclerosis and interstitial fibrosis is a prominent feature of human diabetic nephropathy [1]. There may be an interplay of metabolic and hemodynamic pathways in the progression of diabetic nephropathy [2–4]. For example, the renin-angiotensin system has been postulated to have a pathogenic role in the development of diabetic nephropathy. Angiotensin II, especially, has many nonhemodynamic effects on renal cells that may contribute to the progression of diabetic nephropathy [5]. Angiotensin II induces hypertrophy and/or proliferation of glomerular and tubular epithelial cells, stimulates the synthesis of collagen and fibronectin, and finally, reduces extracellular matrix (ECM) turnover [5]. In addition to metabolic and hemodynamic pathways, the infiltration of inflammatory cells such as monocytes/macrophages (Mϕ) into the diseased kidneys is a hallmark of the progression of diabetic nephropathy [6]. Infiltrated Mϕ release lysosomal enzymes, nitrous oxide (NO), reactive oxygen intermediates (ROIs), and transforming growth factor-β (TGF-β), which have been reported to play an essential role in renal damage [4, 7–9]. However, the mechanisms of the infiltration and activation of inflammatory Mϕ in the diseased kidneys remain to be investigated in the pathogenesis of human diabetic nephropathy.

A chemokine, monocyte chemoattractant protein-1 [MCP-1; also termed as monocyte chemotactic and activating factor (MCAF)], was found to be secreted by mononuclear cells and various nonleukocytic cells, including inflammatory fibroblasts, astrocytes, and renal resident cells, including mesangial cells and tubular epithelial cells [10, 11]. Recent studies reveal that MCP-1 plays an important role in the pathogenesis of crescentic formation and progressive tubulointerstitial lesions via Mϕ recruitment and activation in experimental glomerulonephritis models [12–14] and human nephritis [15–17]. However, the role of MCP-1 in the pathogenesis of diabetic nephropathy remains unknown.

Key words: chemokine, diabetes mellitus, kidney, interstitium, transforming growth factor-β, macrophage/monocyte.

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To determine whether locally produced MCP-1 participates in the pathophysiology of human diabetic nephropathy by Mϕ recruitment and activation, we determined the urinary levels of MCP-1 in patients with diabetic nephropathy and investigated the relationship between MCP-1 levels and renal damage.

METHODS

Patients

Twenty healthy subjects (10 males and 10 females; median age, 57.1 years; range, 16 to 82 years) and 45 patients (32 males and 13 females; median age, 61.1 years; range, 41 to 82 years) with type II (non-insulin-dependent) diabetes mellitus were evaluated in this study. Patients with underlying diseases such as other metabolic diseases, liver diseases, pancreatitis, steroid-induced glucose intolerance, and primary or secondary renal diseases, except for diabetic nephropathy, were excluded from this study. Twenty-three patients had pathological diagnoses verified by renal biopsy. Patients were divided into four groups in terms of proteinuria: negative, microalbuminuria (albumin excretion rate between 20 and 200 μg/min), proteinuria (macroalbuminuria, >200 μg/min, up to 3.5 g/day), and massive urinary protein excretion (>3.5 g/day) showing nephrotic syndrome. Microalbuminuria was determined using as immunoturbidimetric method [18]. Ten patients with minimal-change nephrotic syndrome (proteinuria for less than 1 month) and six patients with membranous nephropathy (proteinuria for more than 6 months) had nephrotic range proteinuria (≥3.5 g/day) as disease controls. In addition, urine samples from six hypertensive patients with microalbuminuria were used for the measurement of MCP-1 as nondiabetic disease controls. The patients in this study were chosen consecutively from May 1988 to October 1998 at Kanazawa University Hospital or its affiliate hospitals. Urinary tract infections were ruled out in all cases by means of bacterial cultures, the microscopic findings, or both, because urinary tract infection is associated with increased urinary MCP-1 levels (data not shown). All renal biopsies were performed with the consent of the patients.

Pathological studies

Twenty-three kidney specimens were obtained by renal biopsy. Kidney specimens fixed in 10% buffered formalin (pH 7.2), embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin, periodic acid-Schiff (PAS) reagent, periodic acid silver methenamine (PAM), and mallory-azan were observed under a light microscope. Each specimen contained 10 or more glomeruli. We especially laid stress on those specimens stained with PAM and PAS. The extent of interstitial fibrosis and tubular atrophy was evaluated and graded on an arbitrary scale from 0 to III: grade 0, not noted; grade I, patchy; grade II, zonal; and grade III, diffuse. The severity of nephrosclerosis (arteriolosclerosis) according to hyalin deposits is as follows: grade 0, not noted; grade I, noted, but less than half around the arteriole; grade II, noted more than half around the arteriole; and grade III, noted all around the arteriole. Nodular lesions, exudative lesions, and mesangiolysis were simply shown as to whether they were present in each specimen. The severity of the diffuse lesions of glomeruli was graded on a scale of 0 to IV according to the description by Gellman et al [19].

MCP-1 measurements

Spontaneously voided midstream urine catches were collected on the morning of renal biopsy. Ten milliliters of the each urine specimen were spun at 200 × *g* for five minutes at room temperature to remove cells and precipitates. Serum samples were obtained from patients at the same time. The urinary supernatants and sera were kept frozen at −70°C until measurement. MCP-1 levels were determined by an enzyme-linked immunosorbent assay (ELISA), using a specific murine monoclonal antihuman MCP-1 antibody (clone ME 69) as a capture and a rabbit polyclonal antibody as the second antibody as previously described [16]. The recovery rate was confirmed to be more than 95% up to 3 ng/mL in these ELISA systems. All assays were performed in duplicate. The detection limits of this ELISA system were 40 pg/mL for human MCP-1. Urinary MCP-1 levels were standardized by the amount of creatinine in the urine.

Immunohistochemical studies

The presence of MCP-1 protein was demonstrated immunohistochemically on formalin-fixed, paraffin-embedded tissue sections treated with catalyzed signal amplification system (Dako, Glostrup, Denmark) using the indirect avidin-biotinylated peroxidase complex method with a specific murine monoclonal antihuman MCP-1 antibody (26j2). Normal mouse IgG1, which had been absorbed with both human liver extracts and immunoglobulin, was used as a negative control. In addition, the absorption test was performed using a monoclonal antihuman MCP-1 antibody with excess recombinant MCP-1 as a negative control. MCP-1–positive cells were counted from more than randomly chosen 10 fields under high-power magnification (×200). CD68-positive cells were detected immunohistochemically on formalin-fixed, paraffin-embedded tissue sections treated by proteinase K for five to six minutes at room temperature using murine antihuman macrophage CD68 monoclonal antibody (clone KP1; Dako). CD68-positive Mϕ were counted from all glomeruli and were expressed as the number of positive cells per glomerulus. Mean interstitial CD68-positive Mϕ were counted from more than randomly chosen 10 fields under high-power magnification (×200).

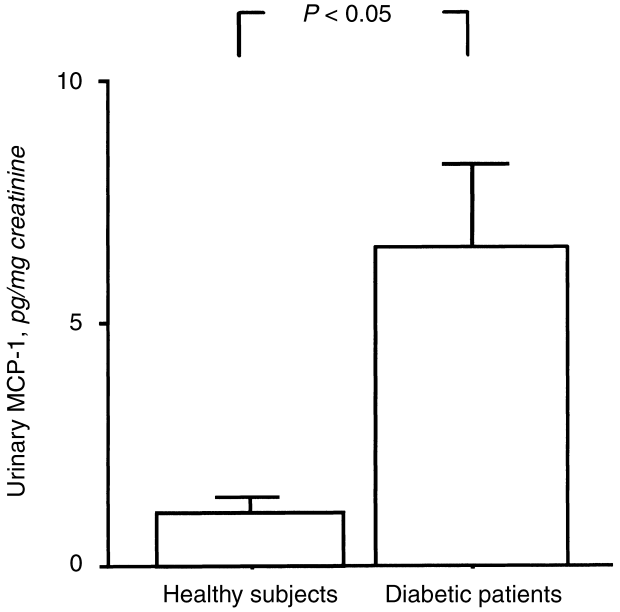

Fig. 1. Urinary monocyte chemoattractant protein-1 (MCP-1) levels in patients with diabetic nephropathy were significantly elevated. Values are mean ± SEM.

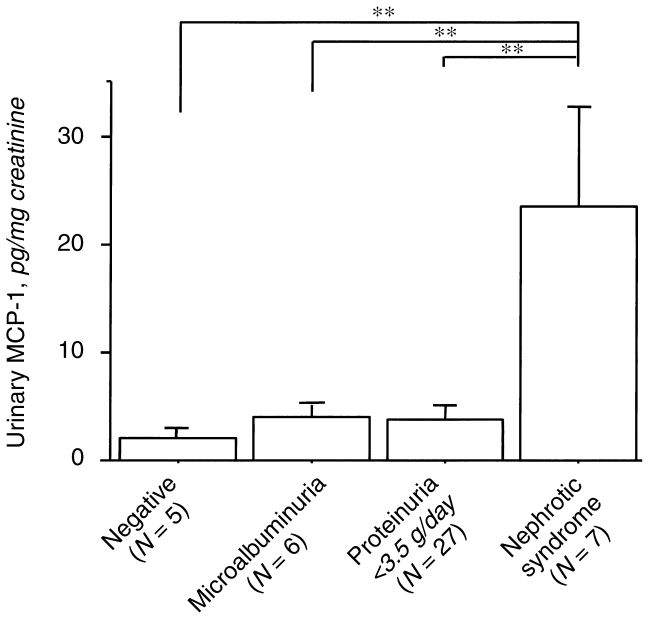

Fig. 2. Urinary levels of MCP-1 increased in patients with massive proteinuria. Values are mean ± SEM. $^{**}P < 0.01$.

In situ hybridization for MCP-1

In situ hybridization procedures were the same as those previously described [16]. A partial sequence of mRNA (5′-CAGAGACTTTCATGCTGGAGGCGAGACTG CGAGCTT-3′) was used for the MCP-1 antisense oligonucleotide probe [20]. Signals were visualized by using commercially available digoxigenin-alkaline phosphatase system (DIG Oligonucleotide Tailing kit and Nucleic Acid Detection kit; Boehringer Mannheim Biochemica, Mannheim, Germany). Negative controls were performed by replacing the antisense probe with a sense probe for MCP-1, which was the exact complement of the antisense probe (5′-AAGCTCGCACTCTCGCCT CCAGCATGAAAGTCTCTG-3′).

Statistics

Values are mean ± SEM. Statistical significance was analyzed using Student's *t*-test, analysis of variance (ANOVA) test, Spearman's and Pearson's correlation coefficient for the analyses of nonparametric and parametric data. $P < 0.05$ was considered to be statistically significant.

RESULTS

MCP-1 levels in diabetic nephropathy

Elevated levels of urinary MCP-1 were detected in the urine sample in patients with diabetic nephropathy (6.5 ± 1.7 pg/mg · creatinine, mean ± MEM) as compared with those of healthy subjects (1.0 ± 0.1 pg/mg · creatinine; Fig. 1). In contrast, serum levels of MCP-1 in diabetic nephropathy were not significantly higher than those of healthy subjects (52.5 ± 12.2 pg/mL vs. 74.0 ± 0.5 pg/mL, respectively). Urinary levels of MCP-1 remained low in patients without protein excretion (1.8 ± 0.2 pg/mg · creatinine, N = 5). Patients with microalbuminuria (3.8 ± 1.2 pg/mg · creatinine, $N = 6$) and patients with proteinuria (3.7 ± 0.5 pg/mg · creatinine, $N = 27$) showed slightly elevated, but not significantly higher levels of MCP-1 as compared with those of healthy subjects or diabetic patients without proteinuria. Patients with massive proteinuria showing nephrotic syndrome showed significantly elevated urinary MCP-1 levels (23.2 ± 9.2 pg/mg · creatinine, $N = 7$) as compared with those of healthy subjects and other diabetic patients (Fig. 2). Furthermore, among the patients with proteinuria and massive proteinuria, urinary levels of MCP-1 significantly correlated with proteinuria ($r = 0.59$, $P = 0.0003$, $N = 34$). Diabetic patients with nephrotic syndrome had significantly severe nephropathy of both glomerular lesions (diffuse lesions, 3.5 ± 0.2) and tubulointerstitial lesions (interstitial fibrosis, 2.5 ± 0.2; tubular atrophy, 2.5 ± 0.2; nephrosclerosis, 2.5 ± 0.2) compared with those of other diabetic patients (diffuse lesions, 1.7 ± 0.2; interstitial fibrosis, 1.1 ± 0.2; tubular atrophy, 1.2 ± 0.3; nephrosclerosis, 1.6 ± 0.3, $P \leq 0.05$, respectively). In contrast, patients with minimal-change nephrotic syndrome showing massive urinary protein excretion as disease controls showed lower levels of MCP-1 in urine (1.6 ± 0.4 pg/mg · creatinine). In addition, patients with membranous nephropathy also showed relatively low levels of urinary MCP-1 (3.9 ± 1.0 pg/mg ·

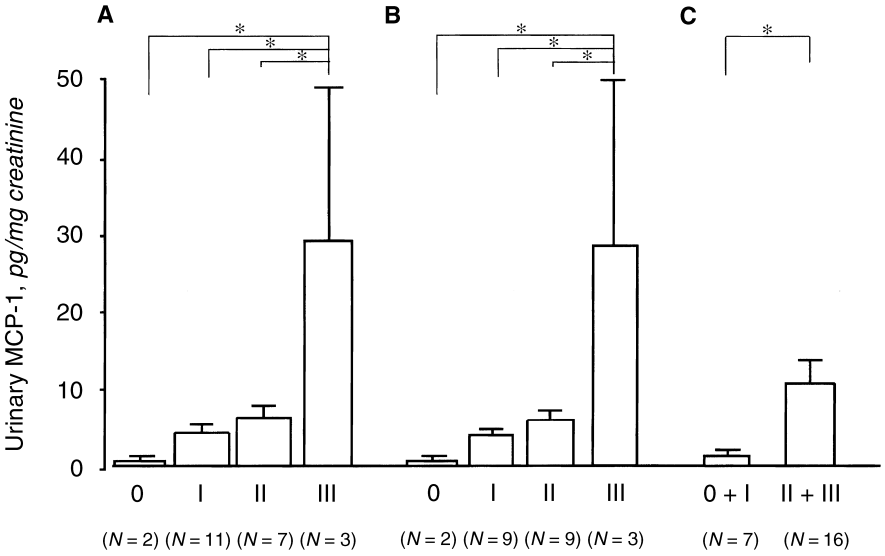

Fig. 3. Correlation of urinary MCP-1 levels and tubulointerstitial lesions in diabetic nephropathy. Urinary levels of MCP-1 increased in accordance with the damage of interstitial fibrosis (*A*) or tubular atrophy (*B*). In addition, there was a significant correlation between urinary MCP-1 levels and the severity of nephrosclerosis (*C*). Values are mean ± SEM. $*P < 0.05$; $**P < 0.01$ by analysis of variance test.

creatinine), which did not give a significant difference from those of healthy subjects. As disease controls in nondiabetic populations, urinary levels of MCP-1 in hypertensive patients with microalbuminuria remained as low as those of normal subjects (1.2 ± 0.4 pg/mg · creatinine, $N = 6$). These results suggest that there might be no correlation between levels of urinary MCP-1 and massive proteinuria showing nephrotic syndrome caused by noninflammatory glomerular capillary lesions.

Correlation of urinary MCP-1 levels and tubulointerstitial lesions in diabetic nephropathy

Urinary levels of MCP-1 increased in accordance with the damage of tubulointerstitial lesions (Fig. 3). MCP-1 levels in urine were significantly elevated in patients with severe interstitial fibrosis (grade 0, 0.9 ± 0.1 pg/mg · creatinine, $N = 2$; grade I, 3.8 ± 0.9 pg/mg · creatinine, $N = 11$; grade II, 6.2 ± 1.6 pg/mg · creatinine, $N = 7$; grade III, 29.0 ± 19.9 pg/mg · creatinine, $N = 3$, $P < 0.05$ when compared with grades 0 to II; Fig. 3A). Similarly, urinary levels of MCP-1 increased in accordance with the severity of tubular atrophy (grade 0, 0.9 ± 0.1 pg/mg · creatinine, $N = 2$; grade I, 3.3 ± 0.8 pg/mg · creatinine, $N = 9$; grade II, 5.6 ± 1.3 pg/mg · creatinine, $N = 9$; grade III, 30.6 ± 19.0 pg/mg · creatinine, $N = 3$, $P < 0.05$ when compared with grade 0 to II; Fig. 3B). In addition, there was a significant correlation between urinary MCP-1 levels and the severity of nephrosclerosis (grades 0 and I, 2.1 ± 0.4 pg/mg · creatinine, $N = 7$; grades II and III, 10.1 ± 4.0 pg/mg · creatinine, $N = 16$, $P < 0.05$; Fig. 3C). Furthermore, there was a significant correlation between the levels of urinary MCP-1 and the number of CD68-positive cells in the interstitium in patients with diabetic nephropathy ($r = 0.63$, $P < 0.05$, $N = 23$).

Correlation of urinary MCP-1 levels and glomerular lesions in diabetic nephropathy

Urinary levels of MCP-1 increased in accordance with the damage of glomerular diffuse lesions, as was observed in the tubulointerstitial lesions (Fig. 4). MCP-1 levels in urine were significantly elevated in patients with severe diffuse lesions (grade I, 2.9 ± 0.9 pg/mg · creatinine, $N = 7$; grade II, 2.6 ± 1.0 pg/mg · creatinine, $N = 6$; grade III, 7.5 ± 1.4 pg/mg · creatinine, $N = 6$; grade IV, 30.5 ± 19.1 pg/mg · creatinine, $N = 4$, $P < 0.05$ when compared with grades I to III; Fig. 4A). In addition, the patients with nodular lesions showed significantly higher levels of urinary MCP-1 than those without them (4.1 ± 0.9 vs. 17.8 ± 10.2 pg/mg · creatinine, $P < 0.05$, $N = 16$ and 7, respectively; Fig. 4B). Furthermore, there was a significant elevation of urinary levels of MCP-1 in patients with exudative lesions as compared with those without exudative lesions (4.6 ± 0.9 vs. 22.1 ± 15.5 pg/mg · creatinine, $P < 0.01$, $N = 18$ and 5, respectively; Fig. 4C). Finally, there was a significant correlation between urinary MCP-1 levels and the presence of mesangiolysis (3.6 ± 0.8 vs. 19.1 ± 9.9 pg/mg · creatinine, $P < 0.05$, $N = 17$ and 6, respectively; Fig. 4D). By comparison, there was no significant correlation between the levels of urinary MCP-1 and the number of CD68-positive cells in the glomerulus in patients with diabetic nephropathy ($r = 0.10$, $P = 0.71$, $N = 23$).

Detection of MCP-1 proteins and transcripts in renal tissues

To determine the local production of MCP-1, renal tissue from 23 patients with diabetic nephropathy was examined via an immunohistochemical as well as in situ hybridization technique for MCP-1. The representative

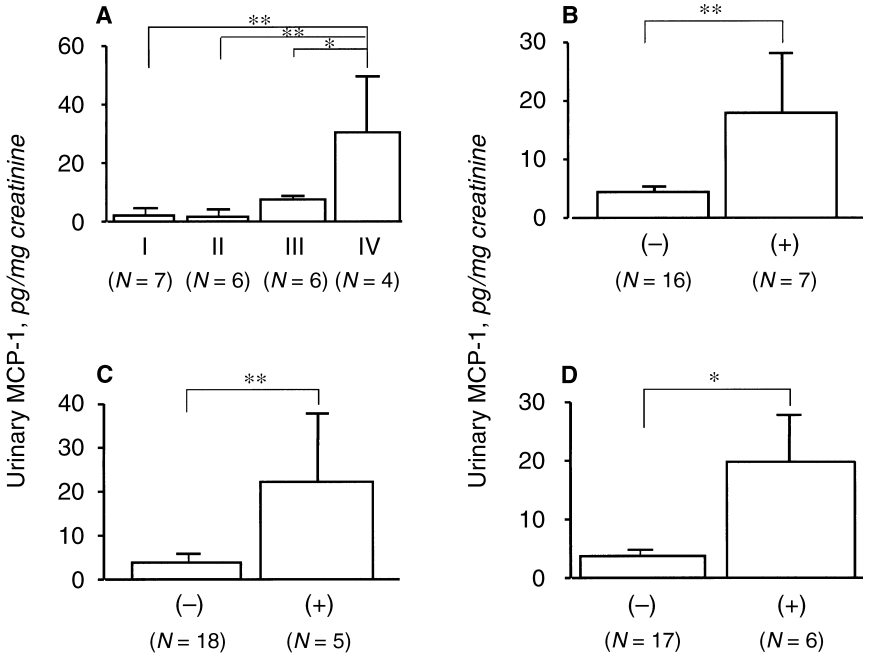

Fig. 4. Correlation of urinary MCP-1 levels and glomerular lesions in diabetic nephropathy. Urinary levels of MCP-1 increased in accordance with the damage of glomerular diffuse lesions (*A*). In addition, the patients with nodular lesions (*B*), exudative lesions (*C*), and the presence of mesangiolysis (*D*) showed significantly higher levels of urinary MCP-1. Values are mean ± SEM. $^*P < 0.05$; $^{**}P < 0.01$ by analysis of variance test.

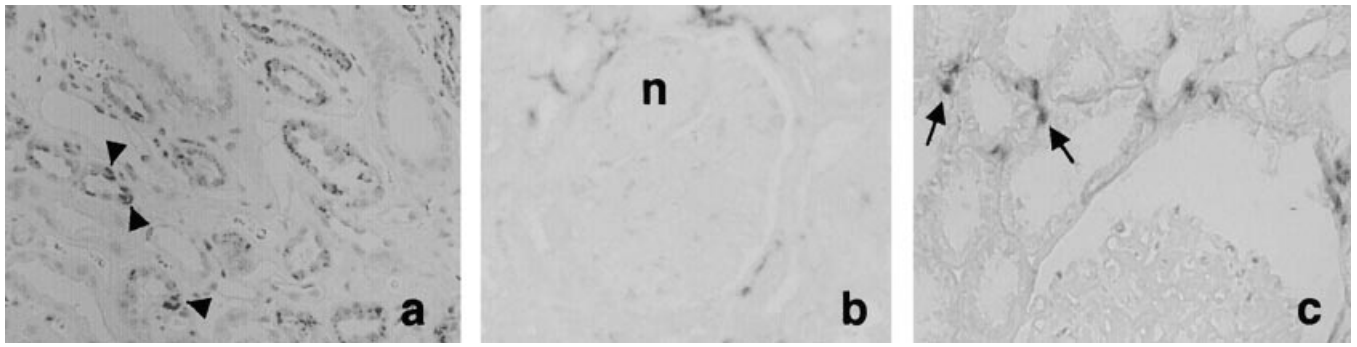

Fig. 5. Detection of MCP-1 in diabetic nephropathy. Expression of MCP-1 protein in renal tissues was detected using a specific monoclonal anti-human MCP-1 antibody as described in the **Methods** section. The sections were observed under a light microscope. The representative case who had advanced diabetic nephropathy [diffuse lesion III, nodular lesion (+), exudative lesion (−), mesangiolysis (−), interstitial fibrosis III, tubular atrophy III, nephrosclerosis II] with nephrotic syndrome (4.2 g/day) was shown here. MCP-1–positive cells were observed mainly in the endothelial, tubular epithelial cells (arrowheads), and infiltrated cells in the interstitium (*a*, ×200). In situ hybridization detected MCP-1 mRNA mainly in cortical tubuli, peritubular capillary endothelial cells, and infiltrated mononuclear cells (arrows) in the interstitium, but it was not detected in the glomeruli (*b* and *c*, ×200; n indicates nodular lesion).

case of a 42-year-old male who had advanced diabetic nephropathy [diffuse lesion III, nodular lesion (+), exudative lesion (−), mesangiolysis (−), interstitial fibrosis III, tubular atrophy III, nephrosclerosis II] with nephrotic syndrome (4.2 g/day) was shown in Figure 5. MCP-1–positive cells were detected in cortical tubuli, peritubular capillary endothelial cells, and infiltrated mononuclear cells in the interstitium in patients with diabetic nephropathy, but was not detected in diseased glomeruli (Fig. 5a). The staining was specific to MCP-1 because neither control isotype-matched murine IgG nor antibody absorbed with recombinant MCP-1 stained positively (data not shown). In addition, the number of MCP-1–positive cells in interstitium (12.3 ± 0.8 per field, $N = 23$) was correlated with urinary levels of MCP-1 ($r = 0.52$, $P < 0.05$, $N = 23$). Furthermore, in situ hybridization detected MCP-1 mRNA signals mainly in cortical tubuli, peritubular capillary endothelial cells, and infiltrated mononuclear cells in the interstitium, but it was not detected in the glomeruli (Fig. 5 b, c). These stainings were specific because positive staining was not detected when the tissues were hybridized with sense oligonucleotide probe for MCP-1 (data not shown).

DISCUSSION

The present study demonstrated that up-regulation of locally produced MCP-1 may be involved in advanced

tubulointerstitial lesions in diabetic patients with nephrotic syndrome possibly through Mϕ recruitment and activation. This concept is based on our findings that (*1*) urinary MCP-1 levels were significantly elevated in patients with diabetic nephrotic syndrome and advanced tubulointerstitial lesions. (*2*) Urinary levels of MCP-1 were correlated with CD68-positive Mϕ in interstitium. (*3*) We detected MCP-1–positive cells in advanced tubulointerstitial lesions of diabetic nephropathy. (*4*) Urinary MCP-1 excretion was correlated with the number of MCP-1–positive cells in the interstitium of renal biopsy specimens. (*5*) Patients with minimal-change nephrotic syndrome or membranous nephropathy showed lower levels of urinary MCP-1, suggesting that massive proteinuria showing nephrotic syndrome did not directly induce elevated levels of urinary MCP-1.

In this study, the expression of MCP-1 was observed to be up-regulated in the interstitium by immunohistochemical and in situ hybridization analyses: tubular epithelial, endothelial cells, and mononuclear infiltrates. In addition, urinary MCP-1 levels were significantly elevated in patients with diabetic nephrotic syndrome and advanced tubulointerstitial lesions. Patients with minimal-change nephrotic syndrome or membranous nephropathy showed lower levels of urinary MCP-1. These data suggest that elevated levels of urinary MCP-1 may reflect advanced tubulointerstitial lesions in diabetic patients. Supporting this notion, we previously reported that MCP-1 plays a pivotal role in the progressive tubulointerstitial damage and promotes renal dysfunction in crescentic glomerulonephritis [15], human lupus nephritis [16], IgA nephropathy [17], and an experimental glomerulonephritis model [12]. Progressive renal diseases have been reported to be associated with tubulointerstitial involvement, and there may be a strict correlation between tubular atrophy, interstitial fibrosis, the extent of interstitial infiltrates, and the renal dysfunction [21]. In addition, interstitial involvement is thought to be the common pathological finding resulting in end-stage renal diseases in spite of causes of renal damages. Thus far, TGF-β has been thought to be the candidate for the key factor to the progression of glomerular and tubulointerstitial lesions, including both nephritis and diabetic nephropathy [22]. A recent study revealed that MCP-1 mediates collagen deposition in experimental glomerulonephritis by TGF-β [23]. In addition, TGF-β may contribute to the secretion of tubular MCP-1 in nephrotic syndrome [24]. Therefore, MCP-1 associated with the up-regulation of TGF-β may be a common pathway involved in the advanced tubulointerstitial damage in diabetic nephropathy as well as in inflammatory renal diseases.

Urinary levels of MCP-1 were elevated in accordance with the progression of diabetic glomerular lesions. However, there was neither the detection of MCP-1 in diseased glomeruli, nor the correlation between the number of CD68-positive cells in glomeruli with urinary levels of MCP-1. Similarly, we previously reported that urinary levels of MCP-1 were well correlated with mesangial proliferation of IgA nephropathy, even though MCP-1 was not detected in the diseased glomeruli by an immunohistochemical study [17]. These results may be derived from the close association between glomerular and tubulointerstitial lesions in each patient of diabetic nephropathy. Supporting this notion, we previously reported that there are three phases in the process of the progression of diabetic glomerulosclerosis: In the first phase, arteriosclerosis and diffuse lesions appear. In the second phase, mesangiolysis and nodular lesions develop in association with moderately advanced arteriolosclerosis, and in the third phase, exudative lesions and hyalinized glomeruli appear in association with advanced arteriosclerosis (nephrosclerosis) together with advanced interstitial lesions [25, 26]. Moreover, in the development of mesangiolysis and layered nodular lesions, disturbed blood flow into glomeruli in the consequence of diabetic arteriolosclerosis (nephrosclerosis) could be essential [25, 26]. Even though there was no direct evidence of MCP-1 expression in glomerular lesions, MCP-1 may have an indirect impact on glomerular lesions via the progression of arteriolosclerosis (nephrosclerosis). Hence, the role of MCP-1 in the pathogenesis of diabetic glomerular lesions requires further investigation.

It is likely that the pathophysiology of diabetic nephropathy involves an interaction of metabolic and hemodynamic factors [4]. Relevant metabolic factors include glucose-dependent pathways such as advanced glycation, increased formation of polyols, oxidant stress, and activation of the enzyme protein kinase C [4, 27]. Supporting this notion, oxidatively modified lipoproteins found in diabetic plasma stimulate MCP-1 gene expression in endothelial cells [28]. In addition, hemodynamic factors include the role of vasoactive hormones, such as angiotensin II. Angiotensin II-dependent pathways to MCP-1 up-regulation have been shown to play an important role in glomerular and tubulointerstitial damage [29–31]. Therefore, these factors, observed in the progression of diabetes mellitus, may stimulate the expression of MCP-1 in the diseased kidneys. Alternatively, massive proteinuria might induce tubular epithelial cells to activate lysosome and antigen presentation followed by the activation of helper T cells in the interstitium [32, 33]. However, at least concerning MCP-1, proteinuria itself did not induce the up-regulation of MCP-1, because patients with minimal-change nephrotic syndrome and membranous nephropathy showing massive proteinuria had lower levels of urinary MCP-1. Taken together, once endothelial cells, tubular epithelial cells, and interstitial infiltrates have been activated by some metabolic and/or hemodynamic process, these cells produce MCP-1, and in turn, MCP-1 may be involved in the progression of advanced tubulointerstitial lesions.

The role of Mϕ in the pathogenesis of diabetic nephropathy remains to be investigated. Thus far, the infiltration of inflammatory cells such as Mϕ into the diseased glomeruli via adhesion molecules may lead to the progression of diabetic nephropathy [6, 34]. This study demonstrated that urinary MCP-1 levels correlated well with the CD68-positive cells in the interstitium as observed in other forms of human nephritis [15, 17]. MCP-1 has been shown to induce the release of lysosomal enzymes and generate superoxide anions, collagen, and TGF-β from Mϕ in addition to being a chemoattractant for Mϕ [21, 35]. In contrast, Mϕ has a scavenging role in the clearance of nonself materials such as altered-self materials, including glycated proteins, oxidized lipoprotein [36]. Even though Mϕ has both effects on renal damage, for example, beneficial effects to prevent renal damage and harmful effects to accelerate renal damage, it is tempting to speculate that interstitial activated Mϕ may play a crucial role in the pathogenesis of advanced tubulointerstitial lesions of diabetic nephropathy. Thus, further investigations are required for establishing the roles of activated interstitial inflammatory Mϕ in the pathogenesis of human diabetic nephropathy.

In summary, our results suggest that MCP-1 may be involved in the pathogenesis of advanced interstitial lesions of diabetic nephropathy as well as inflammatory renal diseases via Mϕ recruitment and activation.

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