Basic research in progressive glomerulopathies: The role of fibrosing factors in IgA nephropathy and diabetic nephropathy

YASUHIKO TOMINO, TOSHIKAO TSUGE, YUSUKE SUZUKI, LEIYI GU, MITSUO TANIMOTO, TOMOHITO GOHDA, and SATOSHI HORIKOSHI

Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan

Renal fibrosis/sclerosis is the common final pathway leading to end-stage renal failure. The renal fibrosis/sclerosis is characterized by increased proliferation of fibroblasts and excessive accumulation of extracellular matrix (ECM). The severity of tubulointerstitial inflammation and fibrosis has been considered to be a crucial determinant of progressive renal injury in patients with various glomerulonephritides. Humoral factors released from infiltrating cells and injured tubular epithelial cells cause further recruitment of inflammatory cells and macrophages and the initiation of fibrogenesis in renal tissues. Human mast cells are a heterogeneous group of multifunctional tissue-dwelling cells with roles in conditions as diverse as allergy, parasite infestation, inflammation, angiogenesis, and tissue remodeling. Immunocytochemical studies have shown the presence within the tissues of two mast cell phenotypes distinguishable by their neutral proteinase content, the tryptase positive mast cell (MCT) phenotype containing only tryptase, and the tryptase and chymase positive mast cell (MCTC) phenotype containing both tryptase and chymase. MCT phenotypes appear to be “immune system–related” mast cells with a primary role in host defense. On the other hand, MCTC phenotypes appear to be “non-immune system–related” mast cells with functions in angiogenesis and tissue remodeling rather than immunologic protection. Recently, we reported the infiltration of tryptase- and/or chymase-positive mast cells in both fibrotic and nonfibrotic areas in the advanced stage of IgA nephropathy [1]. It appears that the number of mast cells in the nonfibrotic areas may be one of the predictive factors for the progression of IgA nephropathy. These mast cells might induce fibrosis and activate angiotensin II. Tubulointerstitial injury was caused by diseases that initially affect the glomeruli or renal vasculatures. Histopathologically, glomerular sclerosis, severe tubulointerstitial fibrosis, and mast cell infiltration in the interstitium were observed in the advanced stage of IgA nephropathy.

Recent findings have shown that monocyte chemoattractant protein-1 (MCP-1), a chemotactic cytokine with a high degree of specificity for monocytes and lymphocytes, may play an important role in the progression of glomerular and tubulointerstitial injuries in experimental and human glomerulonephropathies, including IgA nephropathy and diabetic nephropathy [2]. A variety of cell types, glomerular endothelial cells, mesangial cells, tubular epithelial cells, and monocytes may produce MCP-1 in response to inflammatory signals such as cytokines (IL-1, TNFα, and INFγ) and immune complexes. Interleukin-8 (IL-8), a chemotactic cytokine with a high degree of specificity for neutrophils, is produced by lipopolysaccharide (LPS)-stimulated human peripheral blood monocytes and macrophages, fibroblasts, and endothelial cells, in response to a wide variety of endogenous and/or exogenous stimuli, such as inflammatory cytokines. Among renal resident cells, both glomerular mesangial and proximal tubular epithelial cells can produce IL-8 by proinflammatory stimuli such as LPS, IL-1, and TNFα [3].

IgA nephropathy is well recognized worldwide as one of the most common primary glomerulonephritides, and is characterized by mesangial deposition of IgA in renal specimens [4]. Since the original description by Berger in 1968, several investigations have indicated the developmental and/or exacerbating factors for patients with IgA nephropathy. It is not clear if only IgA deposits in glomeruli are responsible for the glomerular inflammatory changes characteristic of the advanced stage of IgA nephropathy, and the precise mediators signaling lymphocytes and monocytes to migrate and colonize the kidney are not known. However, it is clear that immunoglobulin deposition and/or complement activation has a determinant role. It has been postulated that altered T-cell function may play a major role in the pathogenesis of IgA nephropathy. The authors showed a significant correlation between the levels of serum interleukin (IL)-2 receptor and disease activities [i.e., levels of urinary

Key words: IgA nephropathy, tubulointerstitial fibrosis, diabetic nephropathy.

© 2005 by the International Society of Nephrology
protein, blood urea nitrogen (BUN), and uric acid. We also reported that the high levels of urinary IL-6 reflect the glomerular inflammatory changes in patients with IgA nephropathy [5].

On the other hand, glomerular infiltration of monocytes/macrophages was observed in diabetic nephropathy patients. An increase of MCP-1 expression in the glomerular mesangial areas was also observed in streptozotocin (STZ)-induced diabetic rats [6]. A recent report demonstrated that urinary MCP-1 levels were significantly elevated in patients with diabetic nephrotic syndrome and its advanced tubulointerstitial lesions. Moreover, MCP-1 positive cells were detected in the interstitium of diabetic nephropathy patients [7]. In vitro, high-glucose and glycated albumin has already been reported to facilitate MCP-1 production from human mesangial cells. IL-8 may also be enhanced by high glucose in human endothelial cells and by glycated human serum albumin in human retinal pigment epithelial cells.

**URINARY LEVELS OF MCP-1 OR IL-8 AND RENAL INJURIES IN PATIENTS WITH IGA NEPHROPATHY AND DIABETIC NEPHROPATHY**

Noris et al [8] reported that urinary MCP-1 in patients with active lupus nephritis was significantly higher than in lupus patients studied in the inactive phase of the disease or in healthy volunteers. Using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA), we studied patients with IgA nephropathy to determine if levels of urinary MCP-1 might reflect disease activity. The levels of urinary MCP-1 in patients with the advanced stage of IgA nephropathy were significantly higher than those in patients with the mild stage of the disease, or in healthy control patients [9]. The results showed a significant correlation between the levels of urinary MCP-1 and the disease activity (i.e., levels of urinary casts and urinary protein). Thus, it was suggested that the measurement of urinary MCP-1 is useful in evaluating the degree of renal injuries and/or prognosis in patients with IgA nephropathy.

We also examined the correlation among the levels of urinary MCP-1 and IL-8, hyperglycemia, and renal injuries in patients with type 2 diabetic nephropathy [10]. The levels of urinary MCP-1, IL-8, protein excretion, BUN, serum creatinine (s-Cr), glycohemoglobin A1c (HbA1c), and fasting plasma glucose (FPG) were measured in patients with type 2 diabetic nephropathy and healthy adults as control. Diabetic nephropathy was classified into three stages as follows: stage 1, normoalbuminuric; stage 2, microalbuminuric; and stage 3, macroalbuminuric. All of the patients showed normal ranges in renal function tests (BUN, s-Cr, and CrCr). Levels of urinary MCP-1 in all patients with diabetic nephropathy were significantly higher than those in healthy adults (P < 0.05). The levels of urinary MCP-1 in patients with diabetic nephropathy increased gradually according to the clinical stage of the disease. In contrast, the levels of urinary IL-8 in patients with diabetic nephropathy increased in stages 2 and 3. There was a significant correlation between the levels of urinary IL-8 and those of HbA1c. High glucose may stimulate MCP-1 and/or IL-8 production and their excretion into the urine independently of the disease. It appears that IL-8 increased in the early stage of diabetic nephropathy, and MCP-1 increased in the advanced stage of the disease. It was concluded that measurement of urinary MCP-1 and IL-8 may be useful for evaluating the degree of renal injuries in patients with type 2 diabetic nephropathy [10].

**MCP-1 PRODUCTION ON GLOMERULAR MESANGIAL CELLS AND PODOCYTES**

It is considered that the glomerular and tubular epithelial cells and monocytes may produce MCP-1 in response to some cytokines (IL-1, TNFα, and INFγ), immune complexes, high glucose, or advanced glycation end products (AGEs).

**Glomerular mesangial cells**

Mechanisms of allergic responses have been clarified by extensive studies on FcεR (IgE receptor) and FcγR (IgG receptor). Several studies have clarified that Fc receptors (FcRs) lead to cell activation by multivalent molecules with immunoglobulins, and may play a key role in immunoglobulin-mediated inflammation such as allergic diseases. Moreover, recent studies revealed that FcγR may play an important role in experimental glomerulonephritis using FcγR chain knockout mice. On the other hand, FcγR for IgA (FcγR; CD89) might induce various immunologic responses such as antibody-dependent cellular cytotoxicity (ADCC), and secretion of cytokines and chemokines [11]. FcαR is a glycosylated membrane protein of 50 to 75 kD, which is expressed mainly on monocytes, neutrophils, and eosinophils. Previous studies reported that the FcγR chain is essential for signal transduction via FcγR with IgA in transfected murine B-cell and human monocytic cell lines [12]. A Spanish group reported that FcγR on human cultured mesangial cells might induce cell activation [13], although it is still uncertain whether FcαR activation via the FcγR chain on mesangial cells is actually induced.

Recently, we verified the physical association of FcαR and the FcγR chain on human glomerular mesangial cells and hypothesized their functional expressions [14]. The objective of our study is to investigate whether FcαR (with the FcγR chain) introduced on mesangial cells can be activated and induce phosphorylation of the FcγR chain or syk, and produce the MCP-1 via the mesangial
machinery. Murine mesangial cell lines (SV40 MES 13) were transfected with cDNA of human FcαR. Furthermore, we cotransfected some of the FcαR transfectants with cDNA of the human FcRγ chain. Tyrosine phosphorylation of the intramesangial proteins after FcαR cross-linking was examined by immunoprecipitation. MCP-1 production from each transfectant stimulated with heat aggregated IgA was determined by sandwich ELISA. Two kinds of mesangial transfectants stably expressed human FcαR with or without the FcRγ chain (FcαR+/FcRγ−, FcαR+/FcRγ+). Phosphorylation of the FcRγ chain and syk kinase was detected in FcαR and FcαR+/FcRγ+ cells, but not in untransfected cells. Aggregated IgA induced significantly higher MCP-1 production in FcαR+/FcRγ+ than those in FcαR or the untransfected control. The present study demonstrated that FcαR and the FcRγ chain could be reconstituted in the mesangial cells and mediated MCP-1 production by aggregated IgA in a dose-dependent manner. It appears that FcαR can be activated in mesangial cells through their own machinery, although underlying mechanisms for FcαR induction in mesangial cells remain unclear. It is postulated that IgA immune complexes or aggregated IgA may produce MCP-1 from the mesangial cells under certain conditions in such cells.

**Podocytes**

MCP-1 is a member of the chemokine family regulating macrophage recruitment, and is up-regulated in diabetic nephropathy. Studies using human biopsy materials and animal models have shown the presence of macrophage accumulation and MCP-1 expression in diabetic glomeruli, suggesting that MCP-1 may play an important role in the development of diabetic glomerulosclerosis [15]. Previous studies showed that high glucose and glycated albumin induce MCP-1 production in cultured mesangial cells [16]. However, more recent research found that mRNA expression of MCP-1 appeared to be predominantly localized in podocytes of diabetic glomeruli [15]. We still do not know what induces MCP-1 production by podocytes. Furthermore, intracellular mechanisms of MCP-1 up-regulation in the podocytes remained unclear. Treating db/db mice, a spontaneous diabetes model mouse, with soluble receptor for advanced glycation end products (sRAGE) prevented recruitment of macrophages to the glomeruli, suggesting that ligand receptors for advanced glycation end products (RAGE) may play an important role in MCP-1 production in the podocytes [16]. Expression of RAGE is enhanced in the human diabetic kidney. Specifically, RAGE is expressed at the base of podocytes in glomeruli, but not to any appreciable degree in mesangial or endothelial cells [17]. RAGE, a multiligand member of the immunoglobulin superfamily [18, 19], engages ligands, advanced glycation end products (AGEs), and members of the S100/calgranulin family [20], is implicated in expression of the receptor itself, and amplifies proinflammatory response, leading to diabetic complications and inflammation by a receptor-dependent mechanism [20, 21]. These responses are dependent on RAGE-mediated
signals and expression. It has been shown that RAGE ligand with its ligands results in the activation of multiple signaling pathways in different lines of cells. However, little is known about how ligands, after ligation with RAGE, signal the podocytes involved in diabetic nephropathy through macrophage migration and activation.

Up-regulation of local MCP-1 production is involved in glomerular damage through macrophage recruitment and activation in diabetic nephropathy. Recently, we investigated the role of AGEs in MCP-1 production by podocytes and signaling events after RAGE activation. MCP-1 was induced by AGE and carboxymethyllysine (CML) in a time- and dose-dependent manner as measured by RT-PCR and ELISA. Neutralizing antibody for (CML) in a time- and dose-dependent manner as measured by RT-PCR and ELISA. Neutralizing antibody for AGE suppressed AGE- and CML-induced MCP-1 production completely. Using laser-scanning confocal microscopy, we observed AGE and CML rapidly generated generation of intracellular ROS. NFκB and Sp1 were translocated into the nucleus after podocytes were incubated with CML for 60 minutes. Parthenolide and mithramycin A, inhibitors of NFκB and Sp1, respectively, abolished CML-induced MCP-1 gene expression in a dose-dependent manner. These results suggest that AGE and CML induce MCP-1 expression in the podocytes through activation of RAGE and generation of intracellular ROS. NFκB and Sp1 regulate MCP-1 gene transcription directly (Fig. 1). It appears that the MCP-1 produced by the podocytes in a hyperglycemic state may induce accumulation of monocytes/macrophages and then stimulate the fibrosis in the interstitial regions in patients with diabetic nephropathy (submitted).

Reprint requests to Yasuhiro Tomino, Division of Nephrology, Dept. of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan. E-mail: yasu@med.juntendo.ac.jp

REFERENCES