Monocytic Chemotactic Peptide-1 Expression in Acute and Chronic Human Nephritides: A Pathogenetic Role in Interstitial Monocytes Recruitment

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ABSTRACT

Tubulointerstitial damage is a common histopathological feature of acute and chronic renal diseases and a prognostic indicator of renal function outcome. Monocytes infiltrating the interstitium, through the release of cytokines and/or growth factors, may play a key role in the pathogenesis of tubulointerstitial damage. Monocyte chemotactic peptide-1 (MCP-1) is a specific and powerful chemoattractant and activating factor for monocytes. This study investigated MCP-1 expression and its correlation with monocyte infiltration and tubulointerstitial damage in biopsies of patients with acute interstitial nephritis (AIN) and chronic glomerulonephritis, namely immunoglobulin A nephropathy (IgAN), often characterized by tubulointerstitial involvement. Six patients with AIN and 20 patients with IgAN, nine with mild (G1 to 2) and 11 with moderate to severe histologic lesions (G3 to 5), were studied. MCP-1 gene and protein expression were evaluated by in situ hybridization and immunohistochemistry. Infiltrating CD68-positive cells were identified as monocytes. MCP-1, weakly expressed in normal kidneys, was clearly upregulated in AIN biopsies. The gene and protein expression were primarily localized in tubular and glomerular parietal epithelial cells, as well as in infiltrating mononuclear cells. In IgAN, a striking increase in MCP-1 mRNA and protein expression was observed only in the biopsies with moderate to severe lesions, with a pattern of expression similar to AIN. The MCP-1 expression strictly correlated with monocyte infiltrates and tubulointerstitial damage. In addition, the urinary excretion of this chemokine was studied in 17 IgAN patients. MCP-1 protein concentration was higher, compared with healthy subjects, in IgAN patients, especially in the G3 to 5 group, and directly correlated with the renal MCP-1 gene expression. In conclusion, these data suggest that production of MCP-1 in the tubulointerstitial compartment may play a key role in modulating monocytes influx and, consequently, tubulointerstitial damage.

Key Words: Tubulointerstitial damage, acute interstitial nephritis, IgA nephropathy

Interstitial mononuclear cell infiltration is a common histopathological feature of a variety of acute and chronic renal diseases (1). Indeed, the presence of monocytes in the interstitium has been reported in acute and chronic interstitial nephritis, as well as in chronic glomerulonephritis, and directly correlates with the coexisting histopathological tubulointerstitial changes (tubular atrophy, interstitial fibrosis) (1-3), with the renal function at the time of biopsy, and with the prognosis (4,5). These observations suggest a central role for monocytes in the pathogenesis of tubulointerstitial damage, regardless of the type of primary injury (1). Indeed, these cells, once in the interstitial space, represent a source and/or a reservoir of cytokines and growth factors, promoting and/or maintaining the activation of resident cells (i.e., interstitial fibroblasts and tubular epithelium) (6). Moreover, through the release of oxygen radicals and lysosomal enzymes, monocytes can induce tubular damage, eventually resulting in tubular atrophy and necrosis. The mechanisms involved in the recruitment of monocytes in the interstitium are at present largely unknown, but they should involve the local generation of chemotactic factors. Among these factors, particular interest has been recently focused on monocyte chemotactic peptide-1 (MCP-1) (7). This chemokine is produced, in vitro, by stimulated endothelial, smooth muscle, mesangial, and tubular epithelial cells, as well as by monocytes (8-13). In addition to potently promoting monocyte-specific chemotaxis, MCP-1 activates these cells inducing the respiratory burst and the release of lysosomal enzymes (14). Several investigators have described the expression of MCP-1 in vivo in a variety of diseases associated with a monocyte infiltrate, such as atherosclerosis (15,16), rheumatoid arthritis (17), and idiopathic pulmonary fibrosis (18). Furthermore, there are recent reports on MCP-1 production in vivo at the tubulointerstitial level in experimental models of renal
ischemia, hydronephrosis, and glomerulonephritis (19–21).

In the study presented here, we addressed the possible pathophysiological role of MCP-1 in interstitial monocytes recruitment in acute and chronic human renal diseases. To this purpose, we investigated MCP-1 expression and its correlation with monocyte infiltration and tubulointerstitial damage in biopsies of patients with acute interstitial nephritis (AIN) and with a chronic glomerulonephritis, namely immunoglobulin A nephropathy (IgAN), which is often characterized by tubulointerstitial involvement. Moreover, we investigated the presence of this chemotactic factor in the urine of healthy subjects and of patients with chronic renal disease.

METHODS

Patients

Six patients with primary AIN (mean age, 35 yr; range, 22 to 35 yr) and 20 patients with IgAN (mean age, 25 yr; range, 8 to 46 yr), who had given their informed consent, were enrolled in the study. Diagnosis was made by renal biopsy and standard examination of the cortical tissue by light microscopy and immunofluorescence. IgAN patients with clinical conditions suggesting a secondary glomerulonephritis (systemic lupus erythematosus, liver cirrhosis, Schöönlein-Henoch purpura) were excluded. The histologic diagnosis of IgAN was supplemented by grading of disease severity according to the classification in five classes as described by Lee et al. (22). Conventionally, we indicate G1 to G2 classes as mild and nonprogressive disease and G3 to G5 classes as moderate to severe disease with a potential of progression to end-stage kidney disease.

Specimens

Renal biopsies were performed with a semiautomatic Colt needle (Sterilab, Milan, Italy). Two cortical fragments were obtained from each patient. Immunofluorescence and light microscopy were performed routinely to establish the histologic diagnosis. A portion of the cortical fragment was used for in situ hybridization and immunohistochemistry.

In Situ Hybridization

Renal tissue was immediately included in OCT compound, (Tissue-Tek; Miles Scientific, Elkhart, IN), snap-frozen in liquid nitrogen, and stored in the same liquid until used. Frozen sections (6 μm thick) were collected onto polylysine-coated slides, dried briefly on a hot plate at 80°C and fixed in 4% paraformaldehyde for 20 min. After two washes in phosphate-buffered saline, dehydration in graded ethanol, and brief air-drying, sections were immediately used for in situ hybridization.

For the preparation of the MCP-1 RNA probe, we used a 200-base pair (bp) CDNA template. This template was obtained by reverse transcription-polymerase chain reaction (RT-PCR) of total RNA extracted from cultured human mesangial cells, using two specific primers (sense primer, 5′ TTCTGTCCTGCTGCTCA 3′; antisense primer, 5′ CTCACTCCAAAGTTACCA 3′) deduced from the human full-length CDNA sequence (23). The resulting PCR product was separated by electrophoresis on agarose gel, purified by GeneClean II (Bio 101 Inc., La Jolla, CA), sequenced by Sequenase II (Amersham, Little Chalfont, United Kingdom), and subcloned into the PCRII vector (Invitrogen Corp, San Diego, CA). After linearization of the plasmid with either BamHI or XbaI restriction endonucleases, T7 or SP6 RNA-polymerases (Boehringer, Mannheim, Germany), respectively, were utilized to obtain run-off transcripts of either the antisense (complementary to mRNA) or sense (antisense, negative control) strands. Transcription and labeling of RNA probes was performed as described (24). Briefly, 80 μCi of [35S]uridine-5′-(a-thio)-triphosphate (specific activity, 1250 Ci/mmol; Amersham, Little Chalfont, United Kingdom) was added to a 10-μl reaction mixture (0.5 mM each of adenosine-3′, cytosine-3′, and guanosine-5′-triphosphate/1 mM dithiothreitol/10 U human placental RNase inhibitor/6 mM MgCl2/10 mM Tris-HCl, pH 7.5/2 mM spermidine/10 mM NaN3, including 1 μg of linearized plasmid and 16 U of either SP6 or T7 RNA polymerase. The reaction was allowed to proceed for 60 min at 38°C. The plasmid DNA was removed by digestion with 25 μg/mL RNase-free DNase I in a mixture containing 2.5 mg/mL of yeast tRNA and 10 U of RNase inhibitor for 10 min at 37°C. Free ribonucleotides were removed by phenol-chloroform extraction, followed by ethanol precipitation. RNA probes were then diluted in hybridization buffer, stored at −80°C and used within 4 wk. The specific activity usually obtained was 1.2 to 1.4 × 109 cpm/μg of 35S-labeled RNA probe.

Prehybridization, hybridization, removal of nonspecifically bound probe by RNase A digestion, and further washing procedures were performed for both sense and antisense MCP-1 RNA probe as described previously (24).

Autoradiography was performed by dipping the dehydrated slides into Ilford G5 nuclear emulsion (Ilford, Mobberley Cheshire, United Kingdom). The exposed slides were developed using Kodak D19 developer (Kodak, Hemel Hampstead, United Kingdom), counterstained in hematoxylin and, finally, mounted.

Immunohistochemistry

The immunohistochemical detection of monocytes was performed on frozen 4μm-thick kidney sections using chromatographically purified mouse anti-CD68 monoclonal antibody, specific for human monocytes (Clone EBM11; Dako, Milan, Italy). For immunohistochemical detection of the MCP-1 protein, we used a specific rabbit polyclonal antibaboon MCP-1 antibody, crossreacting with the human peptide (a kind gift of Dr. A.J. Valente, University of Texas at San Antonio). Immunized mouse and rabbit antibodies were detected by the immunalkaline phosphatase (APAAP) method with affinity-purified rabbit anti-mouse and mouse anti-rabbit immunoglobulin serum (Dako) and APAAP complex (1:50 dilution, Dako), following a two- and three-steps technique, respectively, as previously described (24). Alkaline phosphatase was developed with New Fuchsin (Sigma, Milan, Italy). Negative controls were performed by omitting the primary or secondary antibodies, and employing nonimmune mouse or rabbit antisemur as first layer.

Microscopy Studies

Computer morphometry (Optilab Pro 2.6.1 Software, Image Analysis System; Grafeek, Villantero, Pavía, Italy) was used to quantify the histologic lesions and the optical density of the signals generated by silver grains (in situ hybridization). The video image was generated by a video camera (Hamamatsu, Milan, Italy) connected to a Leica microscope (Leitz DMRBE, Wetzlar, Germany) and to a Power PC computer (Macintosh, Cupertino, CA) equipped with a frame
grabber (Hamamatsu). Single images were digitized for image analysis at 256-gray levels. An optical threshold and filter combination was set to select only the nuclei of the infiltrating cells, the area of interstitial fibrosis, and the silver grains. Two observers independently assessed morphological changes, such as tubular atrophy, as well as immunohistochemical signals in a double-blind protocol, as previously described (24).

Total area, number of interstitial CD68-positive cells, areas of interstitial fibrosis, and number of pixels generated by silver grains (in situ hybridization) were quantified in five to eight randomly selected interstitial areas from each biopsy. The regions of interest were interactively discriminated by the operators using the cursor and then automatically measured for total area. Each parameter was calculated by analyzing two serial sections and overlaying the two images obtained. The resulting counts divided by the total area gave the measures desired.

Urinary MCP-1 Assay

Quantitative determination of the MCP-1 protein concentration in the 9-h urine collection of 17 (G1 to G2, 8; G3 to G5, 9) IgAN patients and of eight healthy volunteers was performed using a human MCP-1 ELISA kit (Quantikine R&D, Abingdon, United Kingdom). This is a multiple-sandwich solid-phase enzyme immunoassay, which uses monoclonal antibodies raised against human MCP-1. The sensitivity of the ELISA is 5 pg/mL. The mean recovery of recombinant human MCP-1 spiked in human urine was 92% (range, 85 to 100%). The enzymatic reaction was detected in an automatic microplate photometer (Titertek, Flow Labs, Helsinki, Finland). The MCP-1 concentration of the unknown samples was determined by interpolation into a standard curve developed with known amounts of recombinant human MCP-1 protein. Urine MCP-1 protein levels were normalized to urine creatinine and expressed as pg/mg urine creatinine. Urine samples from AIN patients were not available.

Statistical Analysis

Quantitative data were compared among the groups by analysis of variance. The correlation coefficients are Pearson's r values.

RESULTS

Two groups of patients, characterized by acute or chronic renal damage, were considered. In the first group, we included patients affected by AIN, whereas in the second one we included IgAN patients with mild (nine patients) and moderate to severe histologic lesions (11 patients).

We first investigated the presence of monocytes in the biopsy cores of both groups of patients by immunohistochemistry, using an anti-CD68 antibody, which preferentially recognizes the monocytes coming from the circulation rather than tissue macrophages. In AIN, the interstitial mononuclear cell infiltrate was present in each biopsy, it was diffuse and represented mostly by CD68-positive cells (Figure 1, B through D). The glomeruli were free of specific staining in all of the sections studied (Figure 1, C).

In the second group, represented by IgAN patients, we observed a different pattern of distribution of CD68-positive cells. Monocytes were not constantly present in all of the biopsies studied. Specifically, G1- to G2-graded biopsies showed very few cells positive for the CD68 antigen, either in the interstitium or in the glomeruli (Figure 2, A), whereas the G3- to G5-graded biopsies presented focal areas of CD68-positive cells. Monocytes were not constantly present in all of the biopsies studied. Specifically, G1- to G2-graded biopsies showed very few cells positive for the CD68 antigen, either in the interstitium or in the glomeruli (Figure 2, A), whereas the G3- to G5-graded biopsies presented focal areas of CD68-positive cells.
tive cells infiltrating the interstitium and scattered CD68-positive cells in the glomeruli (Figure 2, B through D). The highest infiltrate density was observed in the periglomerular areas (Figure 2, C). In this second group of patients, the degree of monocyte infiltration strictly correlated with the grade of histologic changes and specifically with the degree of the tubulointerstitial lesions (Table 1).

MCP-1 gene expression in normal and pathological specimens was studied by in situ hybridization, using a specific human RNA probe. In the sections from normal kidneys no specific signal was detected (Figure 3, A and B). On the other hand, in the biopsies of patients with AIN, a diffuse and intense MCP-1 mRNA expression was observed (Figure 3, C and D). No hybridization was seen with the corresponding sense probe (data not shown). The cells expressing MCP-1 were infiltrating mononuclear cells (Figure 4, A and B) as well as glomerular parietal (Figure 3, C and D) and cortical tubular epithelial cells (Figure 4, C and D). When we considered the patients of the second group, we found that while the G1- to G2-graaced biopsy presented very few specific grains (Figure 5, A and B), the biopsies with a significant monocyte infiltration in the interstitium (i.e., G3 to G5) showed a strong signal, when hybridized with the MCP-1 antisense probe (Figure 5, C and D). The chemokine mRNA was again localized in cortical tubular (Figure 6, A and B) and glomerular parietal epithelial cells (Figure 6, C and D), as well as in infiltrating mononuclear cells (Figure 6, A and B) and the expression, just like the monocyte infiltration, showed a focal distribution (Figure 6). We therefore wondered whether the increased mRNA levels for MCP-1 corresponded to an increased protein synthesis. Indeed, as evaluated by immuno-histochemistry using a specific polyclonal anti-baboon MCP-1 antibody, MCP-1 protein expression, absent in normal renal tissue (data not shown), was increased in both groups of patients, with a pattern similar to that observed for mRNA expression (Figure 7). This increased gene and protein expression for MCP-1 in the second group of patients, quantified by a computerized image analysis system, strictly correlated with the number of monocytes infiltrating the interstitium and with the degree of tubulointerstitial damage (Table 1). Moreover, by using serial consecutive biopsy sections, we observed the presence of infiltrating CD68-positive cells mainly, if not exclusively, around those structures whose cells expressed MCP-1 mRNA (Figure 8).

Finally, to evaluate if the increased tissue expression of MCP-1 determined an increase in urinary excretion of this chemokine, we studied the urinary level of MCP-1 in eight healthy subjects, in eight G1 to G2 and in nine G3 to G5 IgAN patients. The urinary concentration of MCP-1 protein was significantly higher, compared with healthy subjects, in both groups of IgAN patients, with a striking increase in the G3 to G5 group (Figure 9). Moreover, the urinary MCP-1 levels directly correlated with the renal MCP-1 gene expression ($r = 0.73, P < 0.05$).

**DISCUSSION**

Tubulointerstitial damage, primarily characterized by tubular atrophy and interstitial fibrosis, is constantly present in progressive renal diseases (25). It is

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**TABLE 1. Correlation among MCP-1 gene expression, monocytes infiltration, and tubulointerstitial damage in biopsies from IgAN patients**

<table>
<thead>
<tr>
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<th>MCP-1 mRNA</th>
<th>Infiltrating Monocytes</th>
<th>Tubular Atrophy</th>
<th>Interstitial Fibrosis</th>
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<td>MCP-1 mRNA</td>
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<td>0.831&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Tubular Atrophy</td>
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<tr>
<td>Interstitial Fibrosis</td>
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<sup>a</sup> $P < 0.001$.  
<sup>b</sup> $P = 0.05$.  
<sup>c</sup> ND, not determined.
now clear that tubulointerstitial, more than glomerular, changes predict the prognosis of human renal diseases (4,5). In the context of tubulointerstitial damage, inflammatory mononuclear cell infiltration is a common phenomenon (25). Although the phenotype of these cells is variable among the different diseases, infiltrating monocytes are almost always present and often represent the primary cell type (1). Several studies in animal models of primary and/or secondary interstitial damage suggest that interstitial monocyte infiltration could be directly linked to the histopathological changes present at the interstitial level (26).

Indeed, interventions aimed at reducing the interstitial influx of monocytes, including systemic irradiation, essential fatty acid-deficient diet, and prednisone treatment, have been shown to attenuate the interstitial fibrosis and the tubular damage in different experimental models of chronic renal diseases (27-29). Once in the interstitial space, monocytes could mediate (through different pathways) the tubulointerstitial lesions (1,6). It is well known that activated monocytes can cause structural tubular damage through the release of proteolytic enzymes and oxygen radicals (28). Moreover, these cells can also promote extracellular matrix deposition through the release of fibrogenic growth factors, such as trans-

Figure 4. In situ hybridization for MCP-1. Dark (A, C) and bright (B, D) field photomicrographs of sections from biopsies of patients affected by AIN. The signal is mainly localized within the mononuclear cells infiltrating the interstitial areas (A, B: original magnification, ×200) and the tubular cells (C, D: original magnification, ×200).

Figure 5. In situ hybridization for MCP-1. Dark- (A, C) and bright (B, D)-field photomicrographs of biopsy specimens from patients with G1 to G2 (A, B) and G3 to G5 (C, D) IgAN. In G1 to G2, MCP-1 expression is very low or completely absent. In G3 to G5, there is a marked increase in MCP-1 message primarily localized, as in the AIN, at the tubulointerstitial level (original magnification, ×200).

Figure 6. In situ hybridization for MCP-1. Dark- (A, C) and bright (B, D)-field photomicrographs of sections from G3 to G5-graded biopsies of IgAN patients. Note the collection of grains, representing the MCP-1 message, within the tubular and parietal epithelial cells as well as the infiltrating cells. The infiltrate is primarily, if not exclusively, present in the proximity of the structures expressing MCP-1 (original magnification, ×200).

Figure 7. Immunoperoxidase staining for MCP-1 of human renal biopsies specimens from patients with AIN (A) and IgAN (B, C). (D) Section stained with nonimmune rabbit anti-serum (original magnification, ×200).
forming growth factor β and platelet-derived growth factor, and specific collagenase inhibitors (6,30). In
line with these concepts, we observed a strict correlation between magnitude of interstitial CD68 positive
cell infiltration and the degree of tubulo-interstitial lesions in the study presented here.

However, although several lines of evidence suggest that monocytes could play a key role in the pathogenesis of tubulointerstitial lesions, the chemotactic factors responsible for their influx and activation at the interstitial level are presently largely unknown. A

great number of chemoattractants have been recently identified. These proteins, including CINC, IP-10, 

RANTES, and MCP-1, are grouped into the small cytokines/intercrine/Scy superfamily (31). Among

these factors, MCP-1 is one of the most extensively studied (32). This chemokine is a powerful and spe-
cific chemotactic factor for monocytes and, in addition to stimulating chemotaxis, it activates these cells,

inducing calcium influx, respiratory burst, adhesion molecule expression, and cytokine production (14,33).

Recently, few studies on experimental models of primary and secondary interstitial damage have sug-
gested a role for this chemokine in monocyte recruitment into the interstitium. In unilateral ureteral 

obstruction, MCP-1 expression was noted in the cortical tubules as early as 12 h after ureteral ligation, in

temporal correspondence to the surge in interstitial macrophage number (20). In a model of primary tu-

bulointerstitial nephritis induced in brown Norway rats by immunization with bovine tubular basement

membrane, MCP-1 expression increased over the first days after the induction, reaching a peak at Day 7 and

shortly preceding the monocyte influx in the interstitium (34). Finally, Eddy and Warren have described a

significant increase in renal MCP-1 mRNA in the puromycin aminonucleoside nephrosis in the rat, a

model characterized by interstitial fibrosis and monocyte infiltrate (21).

Moving from the animal models to the human diseases, there is no information available to confirm the

Figure 8. Serial sections of a biopsy from an AIN patient hybridized for MCP-1 (bright field and dark field, A and B, respectively) and stained for CD68 (C). Note the signal in the

tubular cells and the diffuse CD68 staining around the expressing structures (original magnification, ×400).

Figure 9. MCP-1 urinary level in eight healthy subjects, eight G1 to G2 and nine G3 to G5 IgAN patients, measured by ELISA as described in Material and Methods. Data are represented as mean ± SE. * P < 0.05 versus Control subjects; # P < 0.01 versus G1 to G2.
Experimental reports. Thus, to fill this gap, we decided to investigate MCP-1 gene and protein expression at the tubulointerstitial level in acute and chronic human nephritides and to evaluate its possible relationship with interstitial monocytes infiltration. To this aim, we studied two different pathological conditions, AIN and IgAN, which are characterized by tubulointerstitial lesions and interstitial mononuclear cell infiltration. In AIN, this infiltration was diffuse and present in each biopsy, whereas in IgAN the infiltrating cells were present with a focal distribution only in the biopsies with moderate to severe histologic lesions. In both groups, we demonstrated, for the first time, an increased MCP-1 gene and protein expression that quantitatively correlated with the presence and the extent of monocyte infiltration. Specifically, we observed an increased MCP-1 gene and protein expression only in the biopsies characterized by monocytes infiltration. In addition, MCP-1 expression strictly correlated with the degree of the histologic changes and, particularly, with the severity of tubulointerstitial lesions. Interestingly, besides the simple quantitative correlation between MCP-1 expression and the extent of the interstitial infiltrate, we found that the monocytes were primarily, if not exclusively, localized close to the sites of MCP-1 production: tubular cells, glomerular parietal cells, and infiltrating monocytes themselves. The finding of an increased MCP-1 expression in the infiltrating mononuclear cells is in agreement with the report from Yla-Herttuala et al. (35) on MCP-1 expression in human atherosclerotic lesions and suggests a role for the infiltrating cells in amplifying and/or maintaining the local immune-inflammatory response. This observation, however, would not hamper the role of resident cells in the pathogenesis of the tubulointerstitial damage. Indeed, the observed tubular expression of MCP-1 indicates an active and central role for tubular epithelial cells in the pathological events involving the renal interstitium. According to Ong and Fine, tubular cell injury as a result of chronic ischemia and/or filtered plasma proteins could be crucial in the process of tubulointerstitial fibrosis (36). This injury could lead to the overproduction of growth factors, vasoactive peptides, and chemotactic chemokines such as MCP-1. Generation of MCP-1 by cultured cortical tubular epithelial cells has recently been reported in response to different proinflammatory cytokines (11). Moreover, an increased MCP-1 expression at the tubular level has been observed in two experimental models of tubulointerstitial nephritis (20,21). However, in both these studies, MCP-1 expression was localized only by immunohistochemistry and preferentially at the luminal surface of tubular cells (20,21). These findings may raise some doubts about the tubular production of the chemokine. In the work presented here, using the in situ hybridization technique, we were able to exclude the possibility of a nonspecific uptake of the MCP-1 protein by tubular cells, confirming its local synthesis.

Tubular cells were not the only resident cells expressing high levels of MCP-1 in the biopsies studied. Indeed, glomerular parietal epithelial cells were also a site of MCP-1 expression. Accumulation of monocytes in periglomerular region is a histologic feature of a variety of renal diseases and could potentially affect the structure and the function of the glomerulus (37). It has been proposed that this periglomerular infiltrate could play a role in glomerular sclerosis and crescent formation by causing a disruption of the Bowman's capsule and enabling periglomerular fibroblasts to enter Bowman's space (37). Thus, our finding would suggest a possible pathogenic role for MCP-1 in this phenomenon.

Interestingly, the urinary levels of MCP-1 in the subjects with a chronic renal damage were strictly correlated with the MCP-1 expression at the tubulointerstitial level, as defined by the in situ hybridization and immunohistochemistry. Moreover, the MCP-1 protein concentration in the 9-h urine collection was directly proportional to degree of the tubulointerstitial lesions and of interstitial infiltrate. This observation, once verified in a larger patient population, could suggest the study of the urinary level of this chemokine as a marker of disease activity in the follow up of chronic and progressive renal diseases.

In conclusion, in this study, we have demonstrated the increased MCP-1 gene and protein expression at the tubulointerstitial level both in acute and chronic tubulointerstitial lesions and its correlation with the presence and the extent of monocyte infiltrate. Our observation, fulfilling the second criterion proposed by Abboud (38) to establish the pathogenic role for a cytokine in any pathological process, would suggest a key role for MCP-1 in the pathogenesis of renal interstitial lesions and therefore in the progression of renal diseases. Finally, because the expression of MCP-1 could be modulated in vitro and in vivo by corticosteroids (39,40), this chemokine could also be considered as an easily reachable therapeutic target.

Acknowledgments

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