Increased urinary excretion of monocyte chemoattractant protein-1 during acute renal allograft rejection

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Abstract

Background. Acute rejection is characterized histologically by infiltration of the interstitium by mononuclear cells. Monocyte chemoattractant protein 1 (MCP-1) has recently been identified as a monocyte chemotactic factor. This study examined the possible role of MCP-1 in renal transplantation.

Methods. The concentration of MCP-1 in urine and serum of 19 renal transplant patients was investigated using an inhibition radioimmunoassay. The patients were divided into a non-rejection (NRj) and a rejection (Rj) group. Normal healthy volunteers were included as controls. Immunoperoxidase staining for MCP-1 and CD 14, as a marker for macrophages, was performed in renal biopsies of transplant patients with rejection and six biopsies from histologically normal kidneys, as controls. The size of urinary MCP-1 was determined by gel filtration chromatography and in a number of fractions assessed for monocyte chemotactic activity using a modified Boyden chamber assay.

Results. Urinary excretion of MCP-1 in the Rj group ranged between 250 ng/mmol Cr and 3148 ng/mmol Cr with a median of 612 ng/mmol Cr. This is significantly higher than the results in the NRj group, ranging between 47 ng/mmol Cr and 388 ng/mmol Cr with a median of 229 ng/mmol Cr. In the normal control group, urinary MCP-1 levels ranged between 38 ng/mmol Cr and 74 ng/mmol Cr with a median of 50 ng/mmol Cr. The fractional excretion of MCP-1, calculated on the basis of MCP-1 and creatinine clearances, was found also to be significantly higher in the Rj group as compared to the NRj group. However, there was no significant difference in the serum levels of MCP-1 between the Rj, NRj, and normal control group. The intensity of MCP-1 staining in tubular epithelial cells and the degree of CD14+ cells in the interstitium was significantly higher in renal allograft biopsies than in the normal kidneys. In addition, MCP-1 isolated from urine of renal transplant patients with rejection was filtered with apparent molecular weight of 13 kDa and 11 kDa. Both sizes are chemotactically active for monocytes.

Conclusions. These data suggest that urinary excretion of MCP-1 can be used as a marker for the episodes of acute rejection. The increase of urinary excretion of MCP-1 most likely is the result of local production by tubular epithelial cells. MCP-1 produced locally may, at least in part, be responsible for the influx of macrophages into the interstitium during rejection.

Key words: acute rejection; MCP-1; urinary excretion

Introduction

Acute rejection influences both short- and long-term graft survival of renal allograft [1]. However, non-invasive methods to detect episodes of rejection are still rather unspecific. Histological evaluation of a renal transplant biopsy is therefore still the golden standard for the diagnosis of rejection. Several attempts have been made to develop a simple non-invasive method to detect rejection episodes. Interleukin-2 [2,3], soluble (s) IL-2 receptor [4,5], TNF-α and TNF-α receptors [6], IL-1 [7], sCD23 [8] and neopterin [9] have been reported to be increased in serum of patients with renal transplant rejection. Increased urinary excretion of IL-6 has been reported to predict acute renal allograft rejection [10] to a certain extent.

Histologically, acute renal allograft rejection particularly acute interstitial rejection is associated with infiltration of mononuclear cells in the interstitium of the graft [11–13]. Several factors have been implicated to explain the mechanisms responsible for the infiltration of mononuclear cells into the interstitium such as adhesion molecules and chemotactic factors [14–16]. Monocyte chemoattractant protein-1 (MCP-1) has been recognized as a monocyte-specific chemotactic factor. This chemokine is produced by various cell types from human and animal origin such as mesangial cells, endothelial cells, fibroblasts, and tubular epithelial cells [17–20]. Experimental studies show that MCP-1 plays an important role in the recruitment of
monocytes into the site of injury [21,22]. In a recent study, we have shown that MCP-1 is synthesized de novo by human proximal tubular epithelial cells, and the production of MCP-1 was increased in the presence of inflammatory cytokines IL-1α or TNF-α [23]. In an immunohistochemical study, MCP-1 staining in tubular epithelial cells was shown to be increased in renal biopsies of transplant patients with rejection, although it was not significantly higher than controls [24]. On the basis of these controversial findings we decided to determine whether MCP-1 could play a role in renal transplant rejection. Therefore, we analysed urinary and serum levels of MCP-1 in patients with renal transplantation and whether it could be used as a measure for acute renal transplant rejection.

Subjects and methods

Patients

Twenty consecutive renal transplant patients, transplanted between October 1993 and April 1994 were included in the study. These patients were devided into a group of patients without rejection (NRj) and a group of patients with rejection (Rj). All patients were studied longitudinally following renal transplantation during the first hospitalization. The follow-up in the NRj group was between 10 and 15 days and in the Rj group between 12 and 40 days. Biochemical parameters such as serum creatinine and 24-h urinary excretion of creatinine were performed daily. The clinical data of the NRj and Rj groups are given in Table 1 and 2 respectively. Nine of 20 patients were assigned in the Rj group. The nine patients in the Rj group experienced 16 episodes of acute rejection, which were confirmed by renal biopsy in 12 episodes. One of the 12 episodes of rejection based on histology of renal biopsy, urinary excretion of MCP-1 was assessed the day before, on the day of renal biopsy, and on the day thereafter. The correlation between an increase of urinary excretion of MCP-1 and the severity of acute rejection, as classified according to the Banff classification, was analysed also. In episodes of rejection based on clinical grounds, urinary excretion of MCP-1 was assessed two consecutive days before and the first day of the treatment with methylprednisolone. In the NRj group (11 patients) urinary excretion of MCP-1 was observed on at least 3 consecutive days (average 7 days) during the follow-up with stable serum creatinine and this group was used as a disease control. One patient was not included in the study due to primary non-function of the graft, leading to transplantation at day 4 after transplantation. Twenty urine samples of patients without rejection but with stable serum creatinine after dehospitalization were assessed also for MCP-1 content. As normal control, the levels of MCP-1 were determined in urine from nine healthy volunteers, while serum levels of MCP-1 were determined in 15 subjects. Acute tubular necrosis (ATN) was found in one patient in the NRj group (Table 1, case no. 1) and defined as an acute, potentially reversible decrease in renal function. No ATN was found in the Rj group.

Samples and calculation

Urine samples were obtained from 24-h urine collections in the NRj and Rj groups, and in normal control group. In the NRj and the Rj groups, urine samples were collected daily during the initial hospitalization period. Blood samples from renal transplant patients were taken on the day of urine collection. All urine and serum samples were stored at —20°C until the radioimmunoassay for MCP-1 was performed. The ratio of 24-h urinary excretion of MCP-1 to that of creatinine excretion was calculated in every sample. The values are expressed in nanograms per millimole creatinine (ng/mmol Cr). Fractional excretion of MCP-1 was determined in the NRj and Rj groups and calculated using the formula described for the calculation of fractional excretion of sodium, as follows [25]:

\[ FE_{\text{MCP-1}}(\%) = \frac{U_{\text{M}}}{P_{\text{M}}} \times \frac{P_{\text{Cr}}}{U_{\text{Cr}}} \times 100 \]

where \( FE_{\text{MCP-1}} \) is fractional excretion of MCP-1 (%); \( U_{\text{M}} \) and \( U_{\text{Cr}} \) are 24-h urinary excretion of MCP-1 and creatinine (ng/24 h and mmol/24 h respectively); \( P_{\text{M}} \) and \( P_{\text{Cr}} \) are serum

### Table 1. Clinical data of patients in the non rejection (NRj) group

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>Age</th>
<th>Underlying disease</th>
<th>Donor</th>
<th>Immunosuppression</th>
<th>Complication</th>
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<td>Pred/CsA</td>
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<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>F</td>
<td>32</td>
<td>MPGN</td>
<td>LRD</td>
<td>Pred/Aza</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>65</td>
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<td>LNRD</td>
<td>Pred/CsA</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>26</td>
<td>Glomerulonephrosis</td>
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<td>Pred/CsA</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>61</td>
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<td>Pred/CsA</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>71</td>
<td>Anephric</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>49</td>
<td>Unknown</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
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<td>58</td>
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<td>Pred/CsA</td>
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</tr>
<tr>
<td>10</td>
<td>M</td>
<td>32</td>
<td>ADPKD</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>Ur. Obstr.</td>
</tr>
</tbody>
</table>

*Second transplantation; ADPKD, autosomal dominant polycystic kidney disease; ATN, acute tubular necrosis; Aza, azathioprine; Cd, cadaveric donor; Chr. pyelonephritis, chronic pyelonephritis; CsA, cyclosporin A; LRD, living-related donor; LNRD, living-non-related donor; MPGN, membranoproliferative glomerulonephritis; Pred, prednisone; Ur. Obstr., ureter obstruction.
used for the detection of macrophages. Rabbit anti-mouse IgG and swine anti-rabbit IgG HRP-conjugated antibodies were used for frozen sections as described previously [26].rabbit IgG horseradish peroxidase (HRP) conjugated antibody for 3 h at room temperature. Then 100 ul iodinated-125I-MCP-1 was achieved by adding goat anti-rabbit IgG in 9% polyethylene glycol 6000 in RIA buffer. The samples were subjected to gel filtration on a Superdex H2O, and then applied on a Dowex (mesh 200-400) column modified Boyden chambers as described previously [23].

To determine whether MCP-1 isolated from urine of renal transplant patients with rejection is chemotactically active for monocytes, a number of fractions with known concentrations of MCP-1 were tested in a chemotactic assay using a modified Boyden chambers as described previously [23]. Recombinant human MCP-1 at a concentration of 6 nM was used as a positive control. The results were expressed as a percentage of untreated control.

**Immunohistology**

Nine biopsies obtained from eight patients during rejection (patient no. 9 had two biopsies) were analysed. Therefore renal biopsy specimens obtained from six different kidneys from patients without rejection were available. Therefore under normal conditions the number of infiltrating cells could not easily be established due to cytoplasmic staining of WT14 [14]. The percentage of peritubular CD14+ cells was scored estimating the involved surface-area, because the number of infiltrating cells could not easily be established due to cytoplasmic staining of WT14 [14]. The percentage of peritubular CD14+ cells was scored using 4 point scale: weak intensity (1+); intermediate intensity (2+); and strong intensity (3+) of MCP-1 staining. The number of peritubular CD14+ cells was estimated using the involved surface-area, because the number of infiltrating cells could not easily be established due to cytoplasmic staining of WT14 [14].

**Table 2. Clinical data of patients in the rejection (Rj) group**

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>Age</th>
<th>Underlying disease</th>
<th>Donor</th>
<th>Immunosuppression</th>
<th>Rejection</th>
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<tr>
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<td>Glomerulonephritis</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>52</td>
<td>Intracap. Prolif. GN</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>60</td>
<td>Grawitz tumour</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>23</td>
<td>MPG N</td>
<td>LRD</td>
<td>Pred/CsA</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>61</td>
<td>Membranous Nepr.</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>2</td>
</tr>
<tr>
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<td>M</td>
<td>66</td>
<td>Unknown</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>76</td>
<td>Obstructive urethra</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>53</td>
<td>Salt-losing nephritis</td>
<td>Cd</td>
<td>Pred/CsA</td>
<td>3</td>
</tr>
</tbody>
</table>

*Second transplantation; ATG, antithymocyte globulin; Cd, cadaveric donor; CsA, cyclosporin A; HSPN, Henoch–Schönlein purpura nephritis; Intracap. Prolif. GN, intracapillary proliferative glomerulonephritis; IR, interstitial rejection; LRD, living-related donor; MPG N, membranoproliferative glomerulonephritis; Pred, prednisone; SM, methylprednisolone; VR, vascular rejection.

levels of MCP-1 and creatinine (ng/ml and μmol/I respectively).

**Antibodies**

Recombinant human MCP-1 was purchased from Peprotech, Inc. (Rocky Hill, NJ, USA). Polyclonal rabbit anti-human MCP-1 antibody was raised in our laboratory. The specificity of the antibody was validated as described previously [24]. The CD14 monoclonal antibody WT14 was a gift of Dr W. Tax, Nijmegen [11]. Rabbit anti-mouse IgG and swine anti-rabbit IgG in 9% polyethylene glycol 6000 in RIA buffer. The specificity of the antibody was validated as described previously [24]. The CD14 monoclonal antibody WT14 was a gift of Dr W. Tax, Nijmegen [11]. Rabbit anti-mouse IgG and swine anti-rabbit IgG in 9% polyethylene glycol 6000 in RIA buffer. The intensity of MCP-1 staining in tubular epithelial cells was found to be similar in pattern between diseased and normal kidneys [24]. Therefore, the intensity of MCP-1 staining in tubular epithelial cells was scored using a 3-point scale: weak intensity (1+); intermediate intensity (2+); and strong intensity (3+) of MCP-1 staining. The number of peritubular CD14+ cells was estimated using the involved surface-area, because the number of infiltrating cells could not easily be established due to cytoplasmic staining of WT14 [14]. The percentage of peritubular CD14+ cells was scored using 4 point scale: 1+, 0–25%; 2+, 25–50%; 3+, 50–75%; and 4+, 75–100% of the total peritubular area. Each biopsy was studied in at least five fields with a magnification of 400 × .

**Size of urinary MCP-1**

Portions of 300 ml urine were collected from five different renal transplant patients during rejection, dialysed against water and lyophilized. The samples were dissolved in 6 ml Na-acetate buffer and counterstained with Mayer's haematoxylin.

The distribution of MCP-1 staining in tubular epithelial cells was found to be similar in pattern between diseased and normal kidneys [24]. Therefore, the intensity of MCP-1 staining in tubular epithelial cells was scored using a 3-point scale: weak intensity (1+); intermediate intensity (2+); and strong intensity (3+) of MCP-1 staining. The number of peritubular CD14+ cells was estimated using the involved surface-area, because the number of infiltrating cells could not easily be established due to cytoplasmic staining of WT14 [14]. The percentage of peritubular CD14+ cells was scored using 4 point scale: 1+, 0–25%; 2+, 25–50%; 3+, 50–75%; and 4+, 75–100% of the total peritubular area. Each biopsy was studied in at least five fields with a magnification of 400 × .

**Chemotactic assay**

To determine whether MCP-1 isolated from urine of renal transplant patients with rejection is chemotactically active for monocytes, a number of fractions with known concentrations of MCP-1 were tested in a chemotactic assay using a modified Boyden chambers as described previously [23]. Recombinant human MCP-1 at a concentration of 6 nM was used as a positive control. The results were expressed as a percentage of untreated control.
Urinary MCP-1 in renal transplant rejection

ratio (%) of migrating monocytes into the filter of the sample relative to the number of migrating monocytes in the positive control.

Statistical analysis

Comparison between the groups was performed by using either Student's *t* test for unpaired samples or the Mann–Whitney test when appropriate. To determine the upper limit of normal urinary excretion of MCP-1, the mean ± 2 SD of the samples in the NRj group was taken as the discriminatory level. The correlation between different parameters was calculated from the regression equation. A *P* value < 0.05 was considered significant.

Results

Urinary excretion of MCP-1

In normal controls (*n* = 9), urinary excretion of MCP-1 ranged between 38 and 74 ng/mmol Cr with a median of 50 ng/mmol Cr. In the NRj group (*n* = 10), three values of urinary MCP-1 per patient were chosen during an episode with stable serum creatinine. The values ranged between 47 and 388 ng/mmol Cr with a median of 229 ng/mmol Cr. This result in urinary MCP-1 was not significantly different in 20 urine samples obtained from patients without rejection after dehospitalization. These values ranged between 56 and 368 ng/mmol Cr with a median of 215 ng/mmol Cr. In the Rj group, urinary excretion of MCP-1 ranged between 250 and 3148 ng/mmol Cr with a median of 612 ng/mmol Cr. This was significantly higher than the results in the NRj and in the normal control groups (*P* < 0.001). The urinary excretion of MCP-1 in the normal control, the NRj, and the Rj group is presented in Figure 1. A discriminatory level of 393 ng/mmol Cr was calculated on the basis of the mean ± 2 SD of urinary excretion of MCP-1 in the NRj group using three values per patient. The mean ± 1 SD calculated using three values per patient was 201 ± 96 ng/mmol Cr, and this is not significantly different from the mean of 203 ± 96 ng/mmol Cr calculated using all values in the NRj group during stable renal function, as determined on the basis of serum creatinine levels. When the analysis was restricted to the episodes of rejection based on histology of renal biopsy, it was found that urinary excretion of MCP-1 was increased in nine of 11 episodes (82%) the day before renal biopsy, in seven of 11 episodes (64%) on the day of renal biopsy, and in eight of 11 episodes (73%) the day thereafter. There was a correlation (*r* = 0.52, *P* < 0.05) between the increase of urinary excretion of MCP-1 and the severity of acute rejection, as classified according to the Banff classification.

Figure 2 shows a comparison between the fractional excretions of MCP-1 in the NRj and in the Rj groups. The fractional excretion of MCP-1 in the NRj and in the Rj groups ranged from 1 to 9% and from 5 to 250%, with median of 4 and 22% respectively. The difference between the two groups was statistically significant (*P* < 0.001).

Serum levels of MCP-1

Serum levels of MCP-1 in normal controls (*n* = 15) ranged between 0.5 and 1.4 ng/ml with a median of 0.9 ng/ml. This was not significantly different from the serum levels of MCP-1 in the NRj group (range between 0.4 and 1.9 ng/ml with a median of 0.7 ng/ml) or in the Rj group (range between 0.3 and 2.3 ng/ml with a median of 0.9 ng/ml).

Correlation between urinary excretion of MCP-1 or MCP-1 clearance and serum creatinine or creatinine clearance

No correlation was found between urinary excretion of MCP-1 and serum creatinine in the NRj and Rj groups, while an inverse correlation was observed between urinary excretion of MCP-1 and creatinine clearance in the Rj group (*r* = 0.38, *P* < 0.05).

The clearance of MCP-1 in the Rj group ranged between 0.8 and 15 ml/min with a median of 3 ml/min. It was significantly higher than the clearance of MCP-1 in the NRj (range between 0.6 and 5.3 ng/mmol Cr with median of 2 ml/min). However, no correlation was observed between MCP-1 clearance in the NRj and Rj group and either serum creatinine or creatinine clearance.
Fig. 2. Fractional excretion of MCP-1 in the NRj and Rj group. The mean fractional excretion of MCP-1 in the Rj group is significantly higher than in the NRj. (*P<0.001).

To illustrate the increase of urinary excretion of MCP-1 and serum creatinine in the Rj group, urinary excretion of MCP-1 and serum creatinine levels over a period of time are presented in Figure 3. In seven of 11 episodes of rejection based on clinical and histological signs of rejection, the increase of urinary excretion of MCP-1 was found before the increase of serum creatinine (as indicated by arrow in Figure 3, Pt 1, 2, and 5-9).

The predictive value of the urinary excretion of MCP-1

We calculated the predictive value of the urinary excretion of MCP-1 with respect to the detection of acute rejection, using a discriminatory level of 393 ng/mmol Cr. From a total 42 samples in the Rj group, 27 values were above that level, while in the NRj group all of the values from a total of 79 samples were below the discriminatory level. From this analysis a urinary excretion of MCP-1 above 393 ng/mmol Cr predicts patients who will develop an acute rejection in all cases; however, it does not predict the moment of rejection. A value below the discriminatory level of 393 ng/mmol Cr excluded acute rejection in 84% of the cases.

Immunohistological analysis

The intensity of MCP-1 staining in tubular epithelial cells in the Rj group (mean score 1.8±0.5) was found to be significantly higher (P<0.05) than the intensity of MCP-1 staining in tubular epithelial cells in the control group (mean score 1.0±0). The percentage of CD14+ cells in the interstitium increased with more than 50% of the total peritubular area in seven of nine biopsies, four of them had scores between 75 and 100%. In the other two biopsies the percentage of CD14+ cells was between 25% to 50%. These results
are significantly higher \( (P<0.05) \) than the results in normal kidneys, where CD14\(^+\) cells are found occasionally in the peritubular area (score 1 + , between 0 and 25%). A significant correlation was found between the intensity of MCP-1 staining in tubular epithelial cells and the degree of CD14\(^+\) cells in the peritubular area of renal allograft biopsies \( (P<0.05) \).

**Molecular size and chemotactic activity of urinary MCP-1**

Using gel filtration chromatography, the molecular size of MCP-1 isolated from urine of patients with acute rejection was found to be 13 and 11 kDa. Chemotactic assays performed with the relevant fractions showed that urinary MCP-1 of both sizes were chemotactically active for monocytes. Their activities could be blocked by rabbit anti-human MCP-1 antibody (Figure 4).

**Discussion**

In the present study we found that urinary excretion of MCP-1 in the Rj group exceeded the discriminatory level of 393 ng/mmol Cr, in 71% of the episodes of rejection. In episodes of rejection based on clinical grounds and histology of renal biopsy, the increase of urinary excretion of MCP-1 preceded the increase of serum creatinine in 64% of the cases (Figure 3). In 82% of the episodes of rejection, the increase of urinary excretion of MCP-1 was observed the day before renal biopsy. These findings suggest that increased urinary excretion of MCP-1 can be used as a measure for acute renal allograft rejection. However, more extensive clinical studies are required to confirm this observation.

The results from the present study indicate that urinary excretion and the fractional excretion of MCP-1 were significantly higher in the Rj group compared to the NRj group. Since the fractional excretion was calculated on the basis of MCP-1 and creatinine clearances, this parameter may give a more reliable impression of MCP-1 handling. The increased urinary excretion of MCP-1 could be the result of increased filtration, decreased reabsorption, or increased secretion due to local production of MCP-1 by tubular epithelial cells or leakage from the interstitium. We found that serum levels of MCP-1 in the Rj group were not significantly different compared to the NRj group or the group of normal controls. Because glomerular filtration decreases during rejection episodes, increased filtration of MCP-1 cannot be the explanation. The fractional excretion of around 100% and in some samples even above 100% makes decreased reabsorption less likely. These findings suggest that during rejection, local synthesis of MCP-1 may take place. Indeed, immunohistological analysis showed that the intensity of MCP-1 staining in tubular epithelial cells of renal allograft biopsies is stronger than in controls.

In a previous study, we reported that there was a tendency increased MCP-1 staining intensity in tubular epithelial cells of renal biopsies from transplant patients with rejection; however, the results were not statistically significant [24]. The number of biopsies studied previously were five only, while in the present study nine biopsies were analysed. We think that in the present study, because of the use of nine biopsies, a significance was achieved. We therefore assume that increased urinary excretion of MCP-1 during episodes of acute rejection is most probably the result of tubular production and secretion of MCP-1. However, monocytes/macrophages are also able to synthesize MCP-1, and a possible contribution of monocytes/macrophages MCP-1 in the urine could not be excluded. We found an increase in CD14\(^+\) cells in the peritubular area in association with rejection, a phenomenon which has been observed previously [12]. Based on our previous study that MCP-1 is produced by tubular epithelial cells [23], and that tubular epithelial cells are involved in the process of renal transplant rejection [27,28], the results in the present study indicate that tubular epithelial cells could be responsible for local production of MCP-1. MCP-1 locally produced may induce the influx of macrophages into the interstitium during rejection. However, factors other than MCP-1 should be considered also as mediators responsible for the migration of monocytes into the tubulointerstitial space. Other chemotactic cytokines such as RANTES (regulated upon activation, normal T cell expressed and secreted), macrophage inflammatory protein-1α (MIP-1α), and MIP-1β, members of β chemokine family or C-C chemokines, also function as chemotactants for monocytes as well as for lymphocytes [29–31]. *In-vitro* studies have shown that RANTES is produced by murine tubular epithelial cells [32], and it has been reported recently that RANTES mRNA and protein are detectable in infiltrating mononuclear cells and tubular epithelium during renal allograft rejection [33]. These findings suggest that RANTES has a role in renal allograft rejection. Beside chemotactic factors, adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are known to be involved in inflammatory processes as mediators for the migration of leukocytes [14,15]. The evidence that VCAM-1 and
ICAM-1 expression in tubular epithelial cells is upregulated during rejection [28,34,35] suggests that these molecules play a role in the migration of monocytes into the interstitium during rejection. The fact that the intensity of MCP-1 staining in tubular epithelial cells is correlated well with the degree of CD14+ cells in the peritubular area indicates that MCP-1 may, at least in part, be responsible for the influx of monocytes into the interstitium during rejection. Furthermore, it was shown that urinary MCP-1 isolated from urine of patients with acute rejection is chemotactically active for monocytes. Both sizes of urinary MCP-1, namely the 13 kDa and 11 kDa species, are chemotactically active for monocytes. Other studies using gel filtration chromatography or SDS-PAGE found that MCP-1 derived from different sources range in size between 9 and 17 kDa [36–38]. We observed that radiolabelled recombinant human MCP-1 (rHuMCP-1) migrates as a band of 13 kDa species upon SDS–PAGE analysis under reducing conditions [24].

In conclusion, the present studies suggest that MCP-1 excretion in the urine of renal transplant patients may serve as an acceptable marker for the detection of episodes of acute rejection. The absence of significant changes in serum levels of MCP-1 and the high fractional excretion of MCP-1 suggest that the 13 kDa and 11 kDa species, are chemotactically active for monocytes and infiltrating macrophages are the possible source of this urinary MCP-1. MCP-1 produced locally may, at least in part, be responsible for the infiltration of macrophages into the interstitium during rejection.

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