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ORIGINAL ARTICLE

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Monocyte chemoattractant protein-1 and interleukin-8 levels in children with acute poststreptococcal glomerulonephritis

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Abstract The infiltration of leukocytes into the glomeruli is a major factor in inflammatory glomerular damage in acute poststreptococcal glomerulonephritis (APSGN). Chemokines participate in leukocyte infiltration. The aim of the present study was to investigate the role of monocyte chemoattractant protein-1 (CCL2/MCP-1) and interleukin-8 (CXL8/IL-8) in APSGN with special emphasis on their role in the clinical course of renal disease. Twenty-one children with APSGN were studied. Serum and urinary CCL2/MCP-1 and CXL8/IL-8 levels were measured by ELISA. The relationships between urinary chemokines and the degree of proteinuria were investigated. Serum and urinary CCL2/MCP-1 levels were significantly higher in the acute phase than in the resolution phase and in controls (P <0.05). Urinary CCL2/MCP-1 levels in the control group were significantly lower than in both the acute and resolution phases (P =0.01 and P =0.001, respectively). In the acute phase, urinary CCL2/MCP-1 correlated with the extent of proteinuria (r =0.58, P =0.006) but not with serum CCL2/MCP-1 levels (r =0.21, P =0.36). Urinary and serum CXL8/IL-8 levels were significantly elevated in the acute phase compared with the resolution phase and controls (P <0.05). A consistent increase in urinary CCL2/MCP-1 was found in the acute phase of patients with APSGN, and this correlates with the degree of proteinuria. Our results emphasize the important role of locally produced chemokines in immune-mediated glomerular injury.

Keywords Acute poststreptococcal glomerulonephritis · Interleukin-8 · Monocyte chemoattractant protein-1

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Introduction

Acute poststreptococcal glomerulonephritis (APSGN) is characterized by glomerular hypercellularity due to proliferation of mesangial and endocapillary cells [1]. In the early stage, there is increased non-glomerular cell infiltration consisting of polymorphonuclear leukocytes and monocytes/macrophages. Locally secreted chemokines mediate leukocyte recruitment during the initial and amplification phase of renal inflammation. In turn, the infiltrating leukocytes contribute to the renal damage by releasing inflammatory and profibrotic factors [2].

Chemokines are a large family of cytokines, chemotactic for leukocytes, and are considered to play a crucial role in the recruitment and activation of leukocytes in inflammation. Monocyte chemoattractant protein-1 (CCL2/MCP-1), a specific chemoattractant for monocytes, is implicated in recruiting and activating monocytes/macrophages in the glomerulus in proliferative glomerular diseases [2]. Mesangial cells are a rich source of chemokines, attracting different leukocyte populations into the glomerulus [3].

The increased influx of monocytes and neutrophils induced by CCL2/MCP-1 and macrophage inflammatory protein-2 (CXCL1/MIP-2) could promote increased production of macrophage and neutrophil inflammatory cytokines and oxygen reactive species leading to further tissue damage [4, 5]. Previous studies have focused on the role of CCL2/MCP-1 in renal inflammation and its induction of inflammatory signals [6, 7].

Interleukin-8 (CXL8/IL-8) is a chemotactic cytokine with a high degree of specificity for neutrophils that has been implicated in the pathogenesis of renal inflammation in human glomerulonephritis [8]. We investigated the potential role of CCL2/MCP-1 and CXL8/IL-8 during the clinical course of poststreptococcal glomerulonephritis.

Patients and methods

Patients and controls

Between May 2000 and February 2001, 21 patients with APSGN (9 boys, 12 girls, aged 7.3±2.7 years, range 2.5–14 years) were included in the study within a week of onset of the disease. The patients (n =14) were re-evaluated in the resolution phase (6–8 weeks after onset). Seven had severe proteinuria in the acute phase. The diagnosis of APSGN was based on epidemiological data, clinical presentation, and laboratory findings, including hematuria and proteinuria, C3 level below the normal limit, recent or ongoing β-hemolytic streptococcus group A infection detected by raised anti-streptolysin O (ASO) or anti-DNAse titers (>200 U/ml), and/or a positive throat culture. No clinical or historical evidence of previous renal disease was present. None of the patients had anuria and increased creatinine levels. Ten healthy age- and sex-matched children served as controls. Informed consent was obtained from the parents of the subjects. In the acute phase, patients were classified into two groups according to the severity of proteinuria as follows: group 1 (n =7) urinary protein/creatinine ratio greater than 2, group 2 (n =14) urinary protein/creatinine ratio equal to or less than 2.

Methods

Serum and urine samples were collected both during the acute and resolution phase. Overnight urine samples were collected from all subjects to measure the urinary concentration of CCL2/MCP-1, CXL8/IL-8, and creatinine. Urine samples were stored at -20^0C before testing. Serum and urinary CCL2/MCP-1 levels were measured with a human CCL2/MCP-1 immunoassay kit with a detection limit of 5 pg/ml (quantitative sandwich ELISA, R and D Systems, UK). Serum and urinary CXL8/IL-8 levels were measured with a human CXL8/IL-8 ELISA kit with a detection limit of 5 pg/ml (Biosource International, USA) using a cytoscreen immunoassay method. Urinary CCL2/MCP-1 and CXL8/IL-8 levels were corrected by the urinary creatinine concentration (mg creatinine/dl).

The levels of urinary protein, creatinine, blood urea nitrogen, serum creatinine, total protein, albumin, and C-reactive protein (CRP) and ASO titers were measured by routine laboratory methods. C3 and C4 levels were measured by turbidimetry (DADE Behring, Turbiquant, Germany).

Statistical analysis

Since almost all of the data exhibited a non-normal distribution, statistical analysis of the data was performed by non-parametric analysis. Wilcoxon and Mann-Whitney U tests were performed for comparison of the acute and resolution phases and the controls. Spearmen's non-parametric analysis of correlation was used to calculate the coefficient of correlation between the study variables. The results were expressed as median and interquartile ranges (IQR) and mean±SD for data exhibiting non-normal and normal distributions, respectively. P values less than 0.05 were considered significant.

Results

Clinical characteristics

At the time of admission microscopic or macroscopic hematuria and proteinuria were detected in all patients. Edema and oliguria were present in 15 patients (71%); and 8 patients (38%) had hypertension. In the resolution phase, 14 patients were re-evaluated. There were 6 of group 1 and 5 of group 2 patients with mild proteinuria and microscopic hematuria during the resolution phase. The ASO and CRP values were high in 95% and 42.8%, respectively, during the acute phase and returned to normal in all during the resolution phase. Decreased C3 levels were found in all patients and returned to normal in 5 of 14 patients at 6 weeks and in 9 patients at 8 weeks after onset.

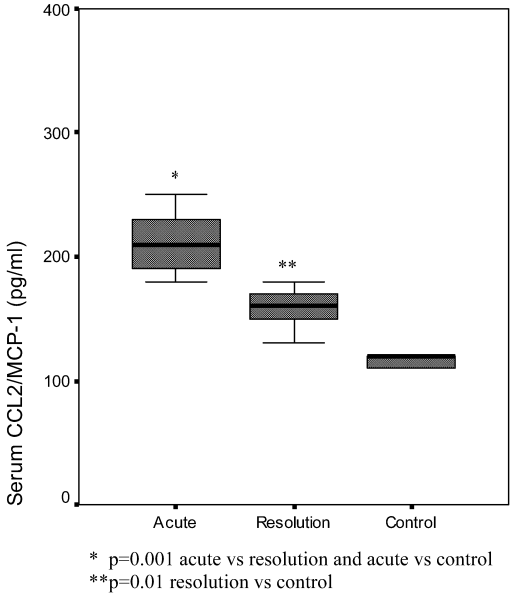
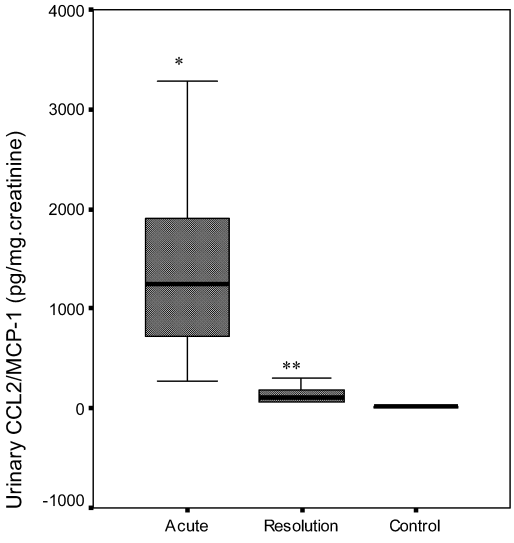


Fig. 1 Serum monocyte chemoattractant protein-1 (CCL2/MCP-1) levels in the acute and resolution phases of the patients with acute poststreptococcal glomerulonephritis (APSGN) and in the healthy controls

The serum CCL2/MCP-1 level was higher in the acute phase [230 (IQR 50) pg/ml] than in the resolution phase [160 (IQR 22.5) pg/ml] and in healthy controls [120 (IQR 10) pg/ml] (P =0.001 and P =0.001, respectively). Furthermore, there was significant difference between the serum CCL2/MCP-1 levels of the patients in the resolution phase and those of the control group (P =0.01) (Fig. 1). In patients with mild-to-moderate proteinuria, serum CCL2/MCP-1 levels were 210 (IQR 80) pg/ml and 155 (IQR 27.5) pg/ml in the acute and resolution phases, respectively. In patients with severe proteinuria serum CCL2/MCP-1 levels during these phases were 235 (IQR 42.5) pg/ml and 165 (IQR 25) pg/ml, respectively. There was no correlation between the severity of proteinuria and serum CCL2/MCP-1 levels (P >0.05).

Urinary CCL2/MCP-1 levels were significantly higher in the acute phase [1,714 (IQR 35.2) pg/mg creatinine] compared with the resolution phase [103.5 (IQR 11. 5) pg/mg creatinine] and the controls [16 (IQR 7.2) pg/mg creatinine] (P =0.001 and P =0.001, respectively). There was also a significant difference between urinary CCL2/MCP-1 levels in the resolution phase and those of the control group (P =0.01) (Fig. 2).



* p=0.001 acute vs resolution and acute vs control
**p=0.01 resolution vs control

Fig. 2 Urinary CCL2/MCP-1 levels in the acute and resolution phases of the patients with APSGN and in the healthy controls

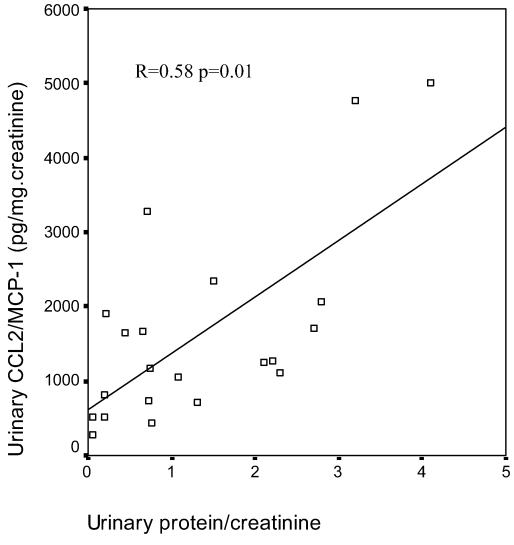


Fig. 3 Correlation between urinary CCL2/MCP-1 levels and the severity of proteinuria in the acute phase

Urinary CCL2/MCP-1 levels in patients with severe proteinuria [1,891 (IQR 36.1) pg/mg creatinine] were significantly higher than those with mild and moderate proteinuria [group 2, 925.5 (IQR 83) pg/mg creatinine] in the acute phase (P =0.04). Urinary CCL/2MCP-1 levels in these patients decreased with resolution [115 (IQR 130) pg/mg creatinine for severe proteinuria and 103 (IQR 111) pg/mg creatinine for mild-to-moderate proteinuria] and the difference between the groups disappeared (P =0.9). Furthermore, urinary CCL2/MCP-1 levels were positively correlated with the severity of proteinuria in the acute phase (r =0.58, P =0.01) (Fig. 3).

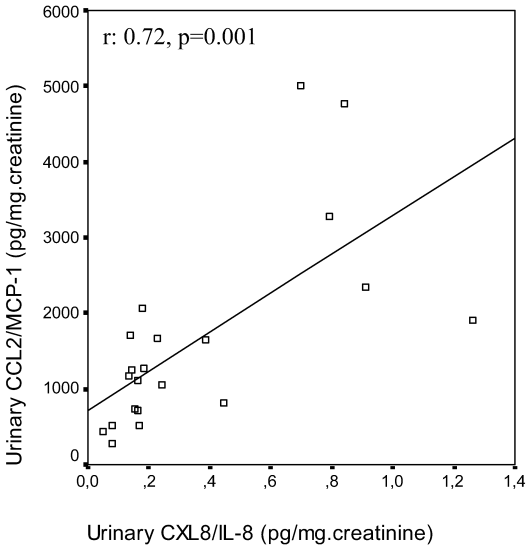


Fig. 4 Correlation between levels of urinary CCL2/MCP-1 and interleukin-8 (CXL8/IL-8) in the acute phase

However, no correlation was found between serum and urine CCL2/MCP-1 levels (r =0.21, P =0.36). In addition, there was no significant difference between the serum CCL2/MCP-1 levels of the patients and severity of proteinuria.

Serum CXL8/IL-8 levels were significantly elevated in the acute phase [0.07 (IQR 0.04) pg/ml] compared with the resolution phase [0.06 (IQR 0.01)] and healthy controls [0.05 (IQR 0.003)] (P =0.001 and P =0.001, respectively). In patients with mild-to-moderate proteinuria, serum CXL8/IL-8 levels were 0.06 (IQR 0.01) pg/ml and 0.07 (IQR 0.02) pg/ml in the acute and resolution phases, respectively. In patients with severe proteinuria these values were 0.07 (IQR 0.05) pg/ml and 0.07 (IQR 0.01) pg/ml, respectively (P >0.05 between groups with severe and mild-to-moderate proteinuria in both acute and resolution phases). Urinary CXL8/IL-8 levels were higher in both the acute and resolution phases [0.18 (IQR 0.56) and 0.08 (IQR 0.04) pg/mg creatinine, respectively] than in the healthy controls [0.002 (IQR 0.004) pg/mg creatinine] (P =0.001 for each). In patients with mild-to-moderate proteinuria, urinary CXL8/IL-8 levels were 0.19 (IQR 0.24) pg/mg creatinine and 0.09 (IQR 0.05) pg/mg creatinine in the acute and resolution phases, respectively. In patients with severe proteinuria these values were 0.17 (IQR 0.6) pg/mg creatinine and 0.07 (IQR 0.01) pg/mg creatinine, respectively (P >0.05 between groups with severe and mild-to-moderate proteinuria in both the acute and resolution phases). There was no significant correlation between urinary CXL8/IL-8 and the severity of proteinuria (r =0.15, P =0.51). Urinary CXL8/IL-8 levels were correlated positively with urinary CCL2/MCP-1 levels in the acute phase (r =0.72, P =0.001) (Fig. 4).

Discussion

Infiltration of leukocyte populations to sites of inflammation is a common feature in APSGN. Chemokines participate in leukocyte infiltration, which plays a major role in glomerular injury during immune complex glomerulonephritis [2]. Chemokines produced by the glomerular cells not only induce recruitment of inflammatory cells but can also alter functions of resident glomerular cells, per se [9]. Therefore chemokines may play an important role in the events leading to podocyte injury and proteinuria.

CCL2/MCP-1 and CXL8/IL-8 have been implicated in recruiting leukocytes to the glomerulus during immune renal injury. CCL2/MCP-1 is both an inflammatory and a chemotactic factor for human mesangial cells [10]. CXL8/IL-8 appears to act as a pro-inflammatory mediator and a chemokine for leukocytes [10, 11].

In this study, urinary CCL2/MCP-1 levels were significantly higher in the acute phase than in the resolution phase or in controls. The origin of elevated urinary CCL2/MCP-1 is not obvious. It might simply reflect increased glomerular permeability. However, the lack of correlation between serum and urinary CCL2/MCP-1 levels in our study suggested an intrarenal source of CCL2/MCP-1. In addition, a positive correlation of urinary, but not serum, CCL2/MCP-1 levels with the degree of proteinuria further supports this observation.

Previous studies indicate that the streptococcal erythrogenic toxin type B (ETB) and its precursor (ETBP) could be involved in the pathogenesis of APSGN [12]. Expression of CCL2/MCP-1 is observed in the mesangium of inflamed glomeruli and can be upregulated in response to streptococcal proteins [13]. The presence of streptococcal proteins in the mesangial microenvironment indicates a possible interaction between ETB-ETBP and intrinsic mesangial cells during APSGN, leading to increased expression of CCL2/MCP-1 and CXCL1/MIP-2 and further influx of neutrophils and monocytes into the renal tissue. Thus it could promote increased production of macrophages, neutrophils, inflammatory cytokines, and oxygen reactive species leading to further tissue damage [13, 14].

Locally secreted chemokines mediate leukocyte recruitment during the initial and amplification phase of renal inflammation. In turn, the infiltrating leukocytes contribute to the renal damage by releasing inflammatory and profibrotic factors [2]. In human and experimental models of glomerulonephritis, MCP-1 plays a pivotal role in the attraction of monocytes into the renal tissue [15, 16]. Anders et al. [17] showed that expression of CCL2/MCP-1 and its receptor precedes proteinuria in a murine model of transient immune complex glomerulonephritis. Their results indicate an important role of CCL2/MCP-1 and its receptor in the early initiation phase of glomerular leukocyte infiltration. CCL2/MCP-1 has also been implicated in the activation of inflammatory cells [18]. We have demonstrated that the degree of proteinuria increases in proportion to the urinary CCL2/MCP-1 excretion. Based on this finding, we have suggested that an increased influx of monocytes and neutrophils through CCL2/MCP-1 expression during the initiation phase could promote increased production of inflammatory cytokines leading to proteinuria that reflects tissue damage.

The neutrophil chemokine CXL8/IL-8 is one of the most intensely studied chemotactic factors in renal diseases. It has been suggested that CXL8/IL-8 may be partially responsible for the infiltration of leukocytes during glomerulonephritis [19]. CXL8/IL-8 is produced mainly by diseased glomeruli and infiltrating cells, and may be involved in the pathogenesis and acute exacerbation of the disease. CXL8/IL-8 is not only a potent chemeoattractant for neutrophils, but also triggers the release of cytotoxic products, which are important for the development of renal damage [11, 20, 21].

In the present study, we found markedly increased urinary CXL8/IL-8 levels independent of serum levels. The lack of correlation between urinary CXL8/IL-8 levels and proteinuria also suggested local production of CXL8/IL-8. However, the levels of urinary CCL2/MCP-1, but not urinary CXL8/IL-8 levels, correlated with the degree of proteinuria. CXL8/IL-8 almost normalized before the disease was in the resolution phase. Therefore, CXL8/IL-8 might play a role in stimulating the disease, whereas CCL2/MCP-1 perpetuates it.

In conclusion, our results suggest that urinary CCL2/MCP-1 and CXL8/IL-8 levels reflect the participation of these chemokines in the glomerular inflammatory response. Furthermore, urinary CCL2/MCP-1 might also be an indicator of the severity of the glomerular inflammatory process. This emphasizes the important role of locally produced chemokines in immune-mediated glomerular injury.

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