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Correlative Insights into Immunoexpression of Monocyte Chemoattractant Protein-1, Transforming Growth Factor β-1 and CD68+ Cells in Lupus Nephritis*

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The experimental data and the study on human renal tissue in patients with glomerulonephropathies indicate that monocyte chemoattractant protein-1 (MCP-1) plays a main role in progression of inflammatory processes in kidney diseases. Monocytes/macrophages are multifunctional cells that may regulate matrix accumulation by producing transforming growth factor β-1 (TGF-β-1), which plays an important role in the progression of renal diseases. The present study was undertaken to evaluate the relationships between the immunoexpression of MCP-1, the number of CD68-positive cells, the immunoexpression of TGF-β-1 and the extent of renal fibrosis as well serum creatinine level in patients with lupus nephritis. Using immunohistochemistry we analyzed the expression of MCP-1, TGF-β-1 and the number of CD68+ cells in renal biopsy specimens in 17 patients with IV class of lupus nephritis and in 10 normal kidneys. Statistical analysis revealed significant increase in the tubulointerstitial MCP-1 immunostaining in lupus nephritis as compared to normal controls. In lupus nephritis the amount of glomerular and interstitial CD68+ cells was higher than in control group. None of the control sections have evidence of glomerular or tubulointerstitial immunoexpression of TGF-β-1. In patients with lupus nephritis TGF-β-1 was detected in the renal tubular epithelial cells and the interstitium, and to a lesser extent within glomeruli. The tubulointerstitial MCP-1 immunoexpression was significantly correlated with monocyte/macrophage interstitial infiltrates, the immunoexpression of TGF-β-1 in tubuli and interstitium as well as serum creatinine. Moreover, the tubulointerstitial immunoexpression of TGF-β-1 was significantly positively correlated with renal interstitial cortical volume and serum creatinine in patients with lupus nephritis. In summary, these data suggest that in lupus nephritis MCP-1 may play a role in modulating interstitial inflammatory process and in tubulointerstitial renal damage *via* TGF-β-1 pathway.

Introduction

The pathogenesis of progressive renal diseases leading to end-stage renal failure is still largely unknown and therefore is a subject of intensive investigation. There is growing evidence that the participation of cellular and inflammatory mechanisms plays a critical role in the progression of renal disease. Tubulointerstitial inflammation comprises an influx and proliferation of inflammatory cells capable of producing a variety of local inflammatory mediators and activation of tubular as well as other intrinsic renal cells. The recruitment of macrophages to sites of acute injury represents a fundamental step in wound healing at any site within the body [9, 20]. However, if these cells persist after the initial damage is repaired, the fibrogenic responses are often sustained with damaging consequences to renal architecture. There is an increasing body of evidence that CC chemokine monocyte chemoattractant protein-1 (MCP-1) plays a major role in the pathogenesis of progression of renal failure [3, 8, 21, 23]. MCP-1 is a major chemoattractant for macrophages. Macrophages are multifunctional cells that are capable of synthesizing a number of secretory products that contribute to ongoing of tissue injury. They may also regulate matrix accumulation, primarily by producing fibrosis promoting growth factors e.g. transforming growth factor-β (TGF-β). The amount of extracellular matrix in the interstitium reflects balance between the production and degradation by proteases. TGF-β contributes to fibrogenesis

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by acting through both pathways. TGF-β directly enhances the synthesis of all major matrix proteins, such as fibronectin, proteoglycans, and collagens. On the other hand TGF-β inhibits matrix degradation by enhancing the production of plasminogen activator inhibitors and enhancing the activity of tissue inhibitors of metalloproteinases [1, 24]. The present study analyzes the immunoexpression of MCP-1 and its relationships with monocyte/macrophage infiltration, the immunoexpression of TGF-β-1, the intensity of renal fibrosis, as well as serum creatinine level in patients with lupus nephritis.

Material and Methods

Patients

Renal tissue biopsies were obtained percutaneously for diagnostic purposes from 17 patients (14 females and 3 males, aged 19–47, mean age=31.4) with lupus nephritis. The duration of SLE before biopsy ranged from 6 months to 12 years, meanwhile the clinical and laboratory presentation of kidney disease ranged from 1 to 14 months. Laboratory data including urinalysis, 24 h protein excretion and serum creatinine level were collected from each patient. At the time of biopsy 6 patients presented nephrotic range proteinuria, in 10 patients proteinuria was more than 2 g/24 h, and in 1 case proteinuria was up to 2 g/24 h. Renal function impairment was noted in 5 patients. In all cases diagnosis of glomerulonephritis was based on characteristic findings by light microscopy (sections stained with hematoxylin and eosin, Masson-trichrome, Jones' silver impregnation and periodic acid-Schiff followed by alcian blue), immunofluorescence and electron microscopy using standard protocols. Classification of the histopathological lesions refers to that of the World Health Organization [2]. In all renal biopsy samples the histopathological changes refer to IV WHO class of lupus nephritis. As a control 10 biopsy specimens from the kidneys removed because of trauma were used. None of the persons from control group were known to have had previous or actual renal disease. Before the quantitative examination was carried out, all control specimens were histologically examined by an experienced nephropathologist and found to be a normal renal tissue.

Immunohistochemistry

Paraffin sections were mounted onto superfrost slides, deparaffinized, then treated in a microwave oven in a solution of citrate buffer, pH 6.0 for 20 min and transferred to distilled water. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide in distilled water for 5 min, and then sections were rinsed with Tris-buffered saline (TBS, Dako Cytomation, Denmark) and incubated with: polyclonal goat anti-human MCP-1 antibody (R&D Systems, dilution 15 μg/ml), polyclonal goat-anti-human TGF-β-1 antibody (Santa Cruz Lab., dilution 1:200) and monoclonal mouse anti-human CD68 antibody (DakoCytomation, Denmark, dilution 1:100). Afterwards LSAB+/HRP Universal kit (DakoCytomation, Denmark) prepared according to the instructions of the manufacturer was used. Visualization was performed by incubating the sections in a solution of 0.5 mg 3,3'-diaminobenzidine (DakoCytomation, Denmark), per ml Tris-HCl buffer, pH 7.6, containing 0.02% hydrogen peroxide, for 10 min. After washing, the sections were counter-stained with hematoxylin and coverslipped. For each antibody and for each sample a positive control and negative control were processed. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results.

Staining intensities of MCP-1 and TGF-β-1 in glomeruli, tubuli and renal interstitium were recorded by two independent observers and graded from 0 (negative), 1 (weakly positive), 2 (moderately positive) and 3 (strongly positive). The mean grade was calculated by averaging grades assigned by the two authors and approximating the arithmetical mean to the nearest unity. Glomerular staining was scored in all glomeruli within renal biopsy specimens. Tubular and interstitial staining was scored in 10 consecutive high power fields, avoiding glomeruli.

Morphometry

Histological morphometry was performed by means of image analysis system consisting of a IBM-compatible computer equipped with a Pentagram graphical tablet, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and color TV camera Panasonic (Japan) linked to a Carl Zeiss microscope (Germany). This system was programmed (program MultiScan 8.08, produced by Computer Scanning Systems, Poland) to calculate the number of objects (semiautomatic function) and the surface area of a structure using stereological net (with regulated number of points). The colored microscopic images were saved serially in the memory of a computer, and then quantitative examinations had been carried out. Interstitial volume in the sections stained with Masson trichrome was measured using point counting method which is an adaptation of the principles of Weibel [26]. The point spacing was 16 μm. The number of the points of a net was 169, and total area was 36864 sq. μm. Under the net described above 8–10 randomly selected adjacent fields of the renal cortex were investigated. Glomeruli and large blood vessels were neglected. The percent interstitial volume was an expression of the num-

ber of points overlying renal cortical interstitium as a percentage of the total points counted.

Quantification of intraglomerular CD68-immunopositive cells: the number of positive cells per glomerular cross-section in each section was obtained (semiautomatic function) and used in the analysis.

Quantification of interstitial CD68-immunopositive cells: interstitial monocytes/macrophages were determined by counting CD68+ cells (semiautomatic function) in a sequence of ten consecutive computer images of 400x high power fields – 0.0047 mm^2 each. The results were expressed as a mean number of CD68-immunopositive cells per mm^2.

Statistical analysis

All values were expressed as the mean ±SD (standard deviation). The differences between groups were tested using Student's t-test for independent samples preceded by evaluation of normality and homogeneity of variances with Levene's test. Additionally the Mann-Whitney U test was used where appropriate. Correlation coefficients were calculated using Sperman's method. Results were considered statistically significant if $P<0.05$.

Results

The data of the glomerular and tubulointerstitial expression of MCP-1, TGF-β-1, a number of CD68-positive cells and value of interstitial cortical volume are shown in Tables 1 and 2. In normal kidneys the immunoexpression of MCP-1 was weak and focal in mesangium, in proximal and distal tubular epithelial cells, renal interstitial cells and interstitial vessel walls. In tissue samples in patients with lupus nephritis the glomerular immunostaining of MCP-1 was mostly related to mesangium and infiltrating inflammatory cells. Statistical analysis did not reveal significant difference between a glomerular MCP-1 immunostaining in normal controls and renal tissue in lupus nephritis. In renal biopsy specimens in lupus nephritis the immunoexpression of MCP-1 was moderate to intense in tubular epithelium, interstitium and interstitial infiltrating cells (Fig. 1). Statistical analysis revealed significant increase in tubulointerstitial MCP-1 immunostaining between renal tissue in lupus nephritis and normal controls ($P<0.001$). None of the con-

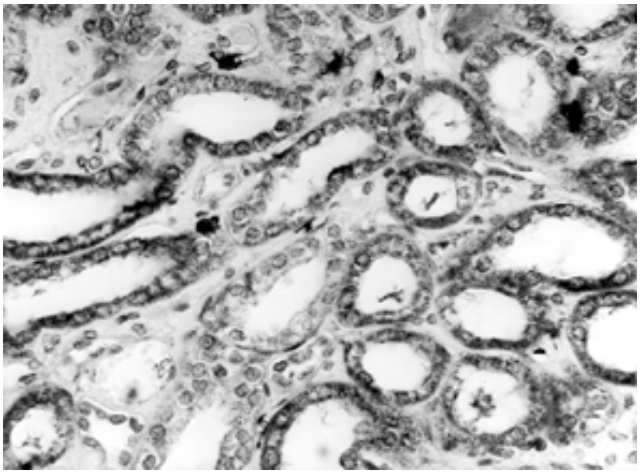

Fig. 1. Moderate immunoexpression of MCP-1 in renal tubular epithelium and interstitial infiltrating cells in patient with lupus nephritis. Magn. 400×.

TABLE 1

The glomerular immunoexpression of MCP-1 and TGF-β–1 and the number of CD68+ cells within glomeruli in patients with lupus nephritis and in normal controls

	MCP-1	TGF-β-1	CD68+ cells
Lupus nephritis (n=17)	0.11±0.13	0.04±0.07	3.36±2.03
Normal controls (n=10)	0.03±0.05	0	1.0±0.6
P value	P=0.08(NS)	P<0.05	P<0.003

TABLE 2

Tubulointerstitial immunoexpression of MCP-1, TGF-β-1, the number of interstitial CD68-positive cells, and interstitial cortical volume in patients with lupus nephritis and in normal controls

	MCP-1	TGF-β-1	CD68+ cells	Interstitial cortical volume
Lupus nephritis (n=17)	1.55±0.9	1.04±0.76	68.91±27.47	20.93±9.31
Normal controls (n=10)	0.38±0.26	0	33.99±18.06	10.9±1.58
P value	P<0.001	P<0.001	P<0.002	P<0.003

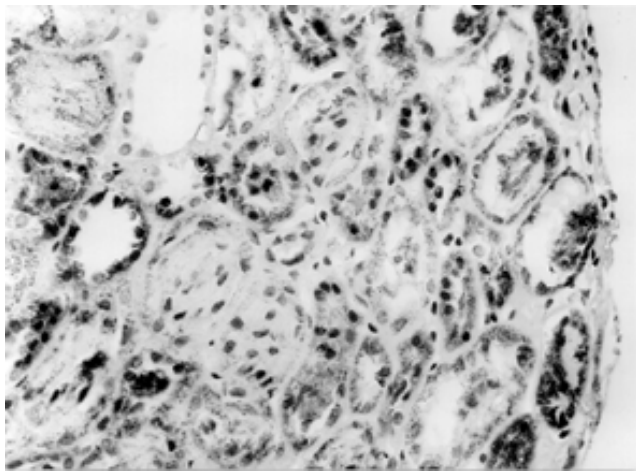

Fig. 2. TGF-β-1 immunostaining in renal tubular epithelial cells in lupus nephritis. Magn. 400×.

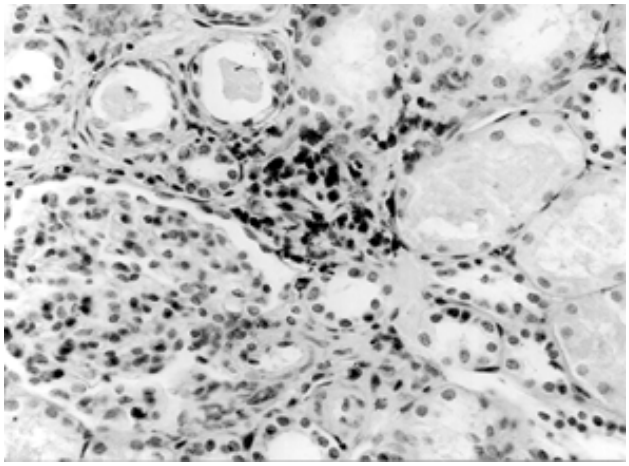

Fig. 3. CD68+ cells in glomeruli and renal interstitium in lupus nephritis. Magn. 400×.

trol sections have evidence of glomerular or tubulointerstitial immunoexpression of TGF-β-1. In renal biopsy specimens in patients with lupus nephropathy TGF-β-1 was detected in the renal tubular epithelial cells and interstitium (Fig. 2), and to a lesser extent within glomeruli. In lupus nephritis the amount of glomerular and interstitial CD68+ cells was higher than in the control group ($P<0.003$ and $P<0.002$, respectively). The interstitial CD68-positive cells were located near glomeruli or atrophic tubules (Fig. 3). There were no correlations between glomerular CD68+ cells, the immunoexpression of MCP-1 and TGF-β-1 in glomeruli in renal biopsy specimens in patients with lupus nephritis (Table 3), meanwhile the tubulointerstitial MCP-1 immunoexpression was positively correlated with monocyte/macrophage interstitial infiltrates ($P<0.005$), immunoexpression of TGF-β-1 in tubuli and interstitium ($P<0.004$) and serum creatinine ($P<0.03$) (Table 4). In lupus nephropathy group the significant positive correlations were found between the tubulointerstitial immunostaining of TGF-β-1,

TABLE 3
The correlations between the glomerular immunomorphological parameters in patients with lupus nephritis

Correlations between:	
MCP-1 and CD68+ cells	r=0.18, P=0.47 (NS)
MCP-1 and TGF-β-1	r=0.34, P=0.17 (NS)
TGF-β-1 and CD68+ cells	r=0.29, P=0.26 (NS)

TABLE 4
The correlations between selected interstitial parameters in patients with lupus nephritis

Correlations between:	
MCP-1 and CD68+ cells	r=0.64, P<0.005
MCP-1 and TGF-β-1	r=0.66, P<0.004
MCP-1 and interstitial cortical volume	r=0.45, P=0.07 (NS)
MCP-1 and serum creatinine	r=0.68, P<0.003
TGF-β-1 and CD68+ cells	r=0.73, P<0.001
TGF-β-1 and interstitial cortical volume	r=0.49, P<0.05
TGF-ß-1 and serum creatinine	r=0.65, P<0.005

interstitial cortical volume ($P<0.05$) and serum creatinine ($P<0.005$) (Table 4).

Discussion

The present study revealed an increase in the tubulointerstitial immunoexpression of MCP-1 in renal tissue specimens in patients with lupus nephritis, meanwhile the intensity of glomerular MCP-1 immunostaining did not differ from normal controls. Similarly to our results, Wada et al. [25] detected MCP-1 gene and protein in infiltrating monocytes and in cortical tubular cells, but surprisingly not in glomeruli in biopsies of systemic lupus erythematosus patients. In experimental study in New Zealand black mice crossed with New Zealand white mice (NZB/W) which serve as a model of lupus nephritis, *in situ* hybridization localized MCP-1 mRNA to intrinsic glomerular cells, infiltrating mononuclear cells and tubular epithelium [27]. Dai et al. [4] who studied an immunoexpression of MCP-1 in renal tissue in patients with lupus nephritis revealed that MCP-1 protein was mainly located at the baso-lateral surface of tubular epithelial cells and on the wall of interstitial blood vessels. It is suggested that an enhancement of MCP-1 staining in tubular epithelial cells may result in part from local production of MCP-1 and the chemotaxis of macrophages towards the tubulointerstitial compartment

may be further stimulated by MCP-1 expression produced by tubular epithelial cells [16]. In our study the number of monocyte/macrophages within glomeruli did not correlate with the glomerular MCP-1 immunostaining, meanwhile the number of CD68-positive cells in interstitium was significantly correlated with the tubulointerstitial MCP-1 expression. These results are in concordance with the study of Prodjosudjadi et al. [16], who found no significant correlation between glomerular MCP-1 staining and glomerular infiltration of macrophages, and they concluded that maybe other factors such as adhesion molecules play an additional role in the infiltration of monocytes/macrophages. MCP-1 is a potent and specific chemotactic and activating factor for monocytes, and is thought to play a major role in recruiting these cells into the site of inflammation [8, 11]. In different forms of glomerulonephritis the strong correlation was found between MCP-1 expression and monocyte/macrophage infiltration supporting a role of MCP-1 in the recruitment of these cells during inflammatory processes [16, 18]. Gesualdo et al. [7] showed a statistically significant up-regulation of MCP-1 gene and protein expression particularly within the areas of tubulointerstitial damage and the glomeruli in cryoglobulinemic membranoproliferative glomerulonephritis. They found a significant correlation between glomerular, as well as tubulointerstitial macrophage infiltration and MCP-1 expression. Monocytes/macrophages infiltrating renal tissue may be a source and/or reservoir of cytokines and growth factors promoting and/or maintaining the activation of resident cells [13, 24]. Infiltrating monocytes/macrophages release lyzosomal enzymes, nitrous oxide, reactive oxygen intermediates and TGF-β, which play an essential role in renal damage. There are several observations in human and experimental glomerulonephropathies correlating the presence of monocyte/macrophages with the histological lesions and with clinical parameters, such as serum creatinine and proteinuria [6, 19]. Our study revealed a strong positive correlation between the tubulointerstitial immunoexpression of MCP-1 and serum creatinine, whereas there was no correlation between MCP-1 and the cortical interstitial volume. These results are in contrary to studies of Ou et al. [15], who suggest that chemokine production in tubulointerstitium is more closely related to the level of urinary protein than to serum creatinine. Urinary excretion of MCP-1 was studied in patients with active and inactive lupus nephritis and was found to be markedly increased during acute lupus nephritis [14, 25]. However, the study of Dai et al. [4] revealed no correlation between MCP-1 production in tubules and the degree of urinary protein excretion in patients with lupus nephritis. These authors showed increased expression of MCP-1 in the renal tubules and vascular wall in patients with lupus nephritis and strong association of MCP-1 with the extent of interstitial fibrosis. The present study reveals the statistically significant positive correlations between tubulointerstitial immunostaining of TGF-β-1, interstitial cortical volume and serum creatinine in patients with lupus nephritis. It is known that TGF-β consists of three isoforms (β1, β2 and β3) and is produced by monocytes and resident cells [17]. TGF-β binds three specific cell-surface receptors, and all of resident cells express at least one of the receptors and respond to TGF-β [20]. The role of TGF-β as a major profibrotic cytokine in various fibrotic diseases in multiple organ systems and in particular in experimental renal disease has been well established [1]. It is though, that extracellular matrix production and/or deposition could be influenced by the presence of MCP-1, either directly as a fibrogenic factor, or indirectly through TGF-β, thus contributing to the development of fibrosis. Mice transgenic for an active form of TGF-β-1 develop progressive renal disease characterized by extracellular matrix accumulation, interstitial fibrosis and proteinuria [10]. Evidence for the central importance of TGF-β in mediating fibrosis in human kidney disease is well supported by the correlation of TGF-β up-regulation with extracellular matrix excess in any type of human kidney disease [22]. Mezzano et al. [12] showed in renal tissue in membranous nephropathy a strong up-regulation of MCP-1, regulated upon activation T cell expressed and secreted (RANTES), osteopontin, TGF-β and platelet derived growth factor (PDGF) mainly in tubular epithelial cells, and an association with the presence of myofibroblasts. It is assumed that the interactions between resident cells (tubuloepithelial and fibroblastic) and infiltrating inflammatory cells (lympho-monocytic) lead to the initiation and progression of tubulointerstitial scarring [9]. Tubular epithelial cells have the capacity to regress from an adult, mature phenotype to an embryonic one in response to injurious stimuli. This so-called transdifferentiation has been reported in response to a variety of growth factors: interleukin-1 (IL-1), epidermal growth factor (EGF) and TGF-β-1 [5].

In conclusion, our results confirmed a pivotal role of MCP-1 in pathogenesis of interstitial kidney damage in lupus nephritis. The mediators of inflammation activate tubular and interstitial cells to produce chemokines, recruitment of inflammatory monocytes/macrophages and activation of growth factors. The strong positive correlations between tubulointerstitial immunoexpression of MCP-1, TGF-β, interstitial monocytes/macrophages, cortical interstitial volume and serum creatinine suggest that in lupus nephritis MCP-1 may play an important role in

modulating interstitial inflammatory process and in tubulointerstitial renal damage *via* TGF-β-1 pathway.

References

1. *Bitzer M, Sterzel RB, Bottinger EP:* Transforming growth factor-β in renal disease. Kidney Blood Press Res 1998, 21, 1–12.
2. *Churg J, Bernstein J, Glassock RJ:* Renal Disease. Classification and Atlas of Glomerular Diseases. Igaku-Shoin. New York, Tokyo 1995, pp151–155.
3. *Cockwell P, Howie AJ, Adu D, Savage COS:* In situ analysis of C-C chemokine mRNA in human glomerulonephritis. Kidney Int 1998, 54, 827–836.
4. *Dai C, Liu Z, Zhou H, Li L:* Monocyte chemoattractant protein-1 expression in renal tissue is associated with monocyte recruitment and tubulo-interstitial lesions in patients with lupus nephritis. Chin Med J 2001, 114, 864–868.
5. *El Nahas M:* Kidney remodeling and scarring: the plasticity of cells. Nephrol Dial Transplant 2003, 18, 1959–1962.
6. *Ferrario F, Castiglione A, Collasanti G, Barbiano di Belgioioso G, Bertoli S, D'Amico G:* The detection of monocytes in human glomerulonephritis. Kidney Int 1985, 28, 513–519.
7. *Gesualdo L, Grandaliano G, Ranieri E, Monno R, Montinaro V, Manno C, Schena FP:* Monocyte recruitment in cryoglobulinemic membranoproliferative glomerulonephritis: A pathogenetic role for monocyte chemotactic peptide-1. Kidney Int 1997, 51, 155-163.
8. *Gomez-Chiarri M, Ortiz A, Seron D, Gonzales E, Egido J:* The intercrine superfamily and renal diseases. Kidney Int 1993, 43 (Suppl 39), S81–S85.
9. *Jernigan SJ, Eddy AA:* Experimental insights into the mechanisms of tubulointerstitial scarring. In: Mechanism and Management of Progressive Renal Failure. El Nahas M, Anderson S, Harris KPG, eds. Oxford University Press, Oxford 2000, pp104–145.
10. *Kopp JB, Factor VM, Mozes M, Nagy P, Sanderson N, Bottinger EP, Klotman PE, Thorgeirsson SS:* Transgenic mice with increased plasma levels of TGF-β1 develop progressive renal disease. Lab Invest 1996, 74, 991–1003.
11. *Leonard EJ, Yoshimura T:* Human monocyte chemoattractant protein-1 (MCP-1). Immunol Today 1993, 11, 97–101.
12. *Mezzano SA, Droguett MA, Burgos E, Ardiles LG, Aros CA, Caorsi I, Egido J:* Overexpression of chemokines, fibrogenic cytokines, and myofibroblasts in human membranous nephropathy. Kidney Int 2000, 57, 147–158.
13. *Nathan CF:* Secretory products of macrophages. J Clin Invest 1987, 79, 319–326.
14. *Noris M, Bernasconi S, Casiraghi F, Sozzani G, Gotti E, Remuzzi G, Mantovani P:* Monocyte chemoattractant protein-1 is excreted in excessive amounts in the urine of patients with lupus nephritis. Lab Invest 1995, 73, 804–809.
15. *Ou ZL, Hotta O, Natori Y, Sugai H, Taguma Y, Natori Y:* Enhanced expression of C chemokine lymphotactin in IgA nephropathy. Nephron 2002, 91, 262-269.
16. *Prodjosudjadi W, Gerritsma JSJ, Van Es LA, Daha MR, Bruijn JA:* Monocyte chemoattractant protein-1 in normal and diseased human kidneys. An immunohistochemical analysis. Clin Nephrol 1995, 44, 148–155.
17. *Roberts WA, Noble NA:* Transforming growth factor-β in tissue fibrosis. N Engl J Med 1994, 331, 1286–1292.
18. *Rovin BH, Rumancik M, Tan L, Dickerson J:* Glomerular expression of monocyte chemoattractant protein-1 in experimental and human glomerulonephritis. Lab Invest 1994, 71, 536–542.
19. *Saito T, Atkins RC:* Contributions of mononuclear leukocytes to the progression of experimental focal glomerular sclerosis. Kidney Int 1990, 37, 1076–1083.
20. *Schena FP, Gesualdo L, Grandaliano G, Montinaro V:* Progression of renal damage in human glomerulonephritides: Is there sleight of hand in winning the game? Kidney Int 1997, 52, 1439–1457.
21. *Schlondorff D, Nelson PJ, Luckow B, Banas B:* Chemokines and renal disease. Kidney Int 1997, 51, 610–621.
22. *Sharma K, Ziyadeh FN:* The emerging role of transforming growth factor-β in kidney disease. Am J Physiol 1994, 35, F829–F842.
23. *Stahl RA:* Chemoattractive cytokines (chemokines) and immune renal injury. Nephrol Dial Transplant 1995, 10, 307–309.
24. *Van Goor H, Ding G, Kees-Folts D, Grond J, Schreiner GF, Diamond JR:* Macrophages and renal disease. Lab Invest 1994, 71, 456–464.
25. *Wada T, Yokoyama H, Su SB, Mukaida N, Iwano M, Dohi K, Takahashi Y, Sasaki T, Furuichi K, Segawa C, Hisada Y, Ohta S, Takasawa K, Kobayashi K, Matsushima K:* Monitoring urinary levels of monocyte chemotactic and activating factor reflects disease activity of lupus nephritis. Kidney Int 1996, 49, 761–767.
26. *Weibel ER:* Stereological Methods. Vol 1. Practical methods for biological morphometry. Academic Press, London, New York, Toronto, Sydney, San Francisco 1979, pp100–161.
27. *Zoja C, Liu XH, Donadelli R, Abbate M, Testa D, Coma D, Taraboletti G, Vecchi A, Dong QG, Rollins BJ, Bertani T:* Renal expression of monocyte chemoattractant protein-1 in lupus autoimmune mice. J Am Soc Nephrol 1997, 8, 720–729.

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