European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2007; 11: 171-178

Urine chemokines: biomarkers of human lupus nephritis?

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Abstract. – Lupus nephritis is characterized by intrarenal inflammation. Leukocytes trafficking from peripheral blood into affected tissues spaces represent an important factor in the development of many renal diseases. During the past few years has been attributed the crucial role of a family of chemotactic cytokines - the chemokines - in this process. In the course of renal diseases, the infiltration of monocytes/macrophages and T cells into kidneys represent an important role in progressive interstitial fibrosis and the progression of chronic renal failure.

In this review, we summarize the in vitro and in vivo data on chemokines and chemokine receptors in kidney diseases, with a special focus on urine chemokine measurement as possible biomarker of human lupus nephritis.

Key Words:

Chemokines, Lupus nephritis, Transforming growth factor-β, Monocyte chemoattractant protein-1, Interleukin-8.

Introduction

Chemokines are a family of chemotactic cytokines that were first identified on the basis of their ability to induce the migration of different cell types[1]. Many studies have demonstrated that chemokines, behaving in concert with selectins and integrins, act as directional signals to effector leukocyte migration and have also been shown to activate leukocytes and modulate angiogenesis[2,3].

The receptors for chemokines are expressed in a cell typespecific manner and are restricted primarily to subsets of leukocytes[4].

Recent advances have included the discovery of new chemokines, receptors, and antagonists, and a greater appreciation for the diverse biologic functions displayed by this cytokine family[4,5]. A wealth of recent data have dealt with the basic biology and the roles of chemokines in various disease processes[1,4,6-8].

Chemokines and Th1/Th2 Immune Responses

The immune responses have been termed Th1-like and Th2-like after the two classes of T helper cells (Th) involved. Th1 responses are stimulated by pathogens that inhabit cells and result in activation of cytotoxic T lymphocytes and delayed-type hypersensitivity. The Th1 subtype produces cytokines, such as IFN-γ, IL-2, leukotriene A, granulocyte macrophage colony-stimulating factor, that stimulate strong cellular immune responses. The Th2 subtypes produce cytokines that induce antibody responses (IL-3, IL-4, IL-5, IL-6, IL-10, and IL-13). Moreover, Th2 cytokines can inhibit the inflammatory reactions induced by Th1 cytokines. A third subtype called Th0 secretes cytokines of both types and is believed to give rise to the "polarized" Th1 and Th2 lineages. Cobbold and Waldmann have characterized Th3/T-regulatory-1 T cell subset demonstrating the down regulation of antigen-presenting cells, possibly via transforming growth factor-β (TGF-β)[9].

Several data suggest that differential chemokine receptor expression may be important for the generation of a Th1- or Th2-type immune response, because specific chemokine receptor expression has been shown to characterize these T helper cell subtypes[10]. Th1 cells appear to preferentially express the chemokine receptors CX-

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CR3 and CCR5, while Th2 cells display CCR4, CCR8, and some CCR3[8,10,11].

Chemokines and Inflammatory Process

Inflammation is a process involving changes in hemodynamics, vascular reaction of endothelial cells, leukocyte adhesion, activation, and migration[12]. The nature of the inflammatory response is dictated by the pathogenic insult. The process of leukocyte trafficking from the peripheral circulation into tissue spaces involves a series of interactions between soluble mediators and surface molecules expressed by the endothelium and leukocyte, as well as subsequent interactions with the extracellular matrix[13].

Activated leukocytes in the site of inflammation can produce additional proinflammatory factors resulting in amplification of the inflammatory response. At the site of tissue injury, the accumulation of specific leukocytes results in removal of the initiating insult (e.g., phagocytosis of bacteria, immune complexes, apoptotic cells, etc.) and tissue repair. Inactivation and removal of inflammatory cells is important to prevent chronic inflammation and progressive tissue destruction.

In the course of renal diseases, the infiltration of monocytes/macrophages and T cells into kidneys play a crucial role in progressive interstitial fibrosis and the progression of chronic renal failure[14]. During this process, a cross-talk between cytokines, vasoactive substances, chemokines, and their respective target cells takes place. This interaction contributes to the outcome, i.e., healing or progression of the renal disorder[15].

All types of renal cells (endothelial, mesangial, tubular epithelial, interstitial cells, and podocytes) can express chemokines upon stimulation. Chemokines contribute to inflammatory glomerular as well as tubulointerstitial diseases[16-24]. Proinflammatory stimuli, such as TNF-α, IL-1, IFN-γ and lipopolysaccharide (LPS), within a few hours, induce monocyte chemoattractant protein-1 (MCP-1) and IL-8[24,20,25]. Reactive oxygen species are able to upregulate chemokine expression and may represent a common mechanism of injury-induced chemokine generation[21].

Mesangial cells after activation can express MCP-1 by growth factors such as platelet growth factor and basic fibroblast growth factor[26,27]. This expression may be related to the macrophage influx observed during proliferative responses related to tissue repair and remodeling. MCP-1 expression by proximal tubular cells is found after exposure to hyaluronan, a glucosaminoglycan degradation product of extracellular matrix that accumulates in the interstitium during kidney diseases[28]. The CD40 interaction with its ligand (CD154), together with IL-4 and IL-13, results in MCP-1 and IL-8 generation by cultured proximal tubular cells[29]: this represent a relevant datum for tubulointerstitial inflammatory cell infiltrates in transplant rejection and other types of interstitial diseases.

The chemokines expression by proximal tubular cells can be induced by albumin, which is thought to mimic the effects of proteinuria and may be related to the tubulointerstitial damage observed in glomerular disease[30]. Figure 1 represents a model of potential role for chemokines in different pathophysiologic processes thought to contribute to renal injury.

Urine Chemokines and Human SLE Nephritis

Many studies have demonstrated that chemokines and chemokine receptors play an important role in glomerular and tubulointerstitial diseases[31-34], in proliferative and crescentic glomerulonephritis[35,36] and during renal transplant rejection[37,38].

Recent developments suggest that monitoring chemokines in the urine may provide a more dynamic picture of the inflammatory state of the kidney[39].

Wing-Yan Chan et al. examined the gene expression of transforming growth factor-β (TGF-β) and MCP-1 in the urinary sediment of SLE patients to evaluate the possible interrelationship between the urinary expression of TGF-β and MCP-1 mRNA and their corresponding protein levels in urine, as well as their association with kidney involvement in these patients[40]. The Authors studied 3 groups of subjects: all of them fulfilled the American College of Rheumatology diagnostic criteria for SLE[41]. Active lupus was defined as an SLE Disease Activity Index (SLEDAI) score of ≥ 6[42,43]. Forty-nine patients with SLE displaying systemic disease activity and renal involvement were enrolled (designated the active disease group). Renal involvement was defined as a 150% increase in the 24 hour measurement of total urinary protein levels, a 120% increase in the serum creatinine levels, presence of pyuria (≥ 6 leukocytes/highpower field, presence of hematuria (≥ 5 red blood cells/high power field), or presence of urinary casts by microscopic examination of urinary sediment. Were al-

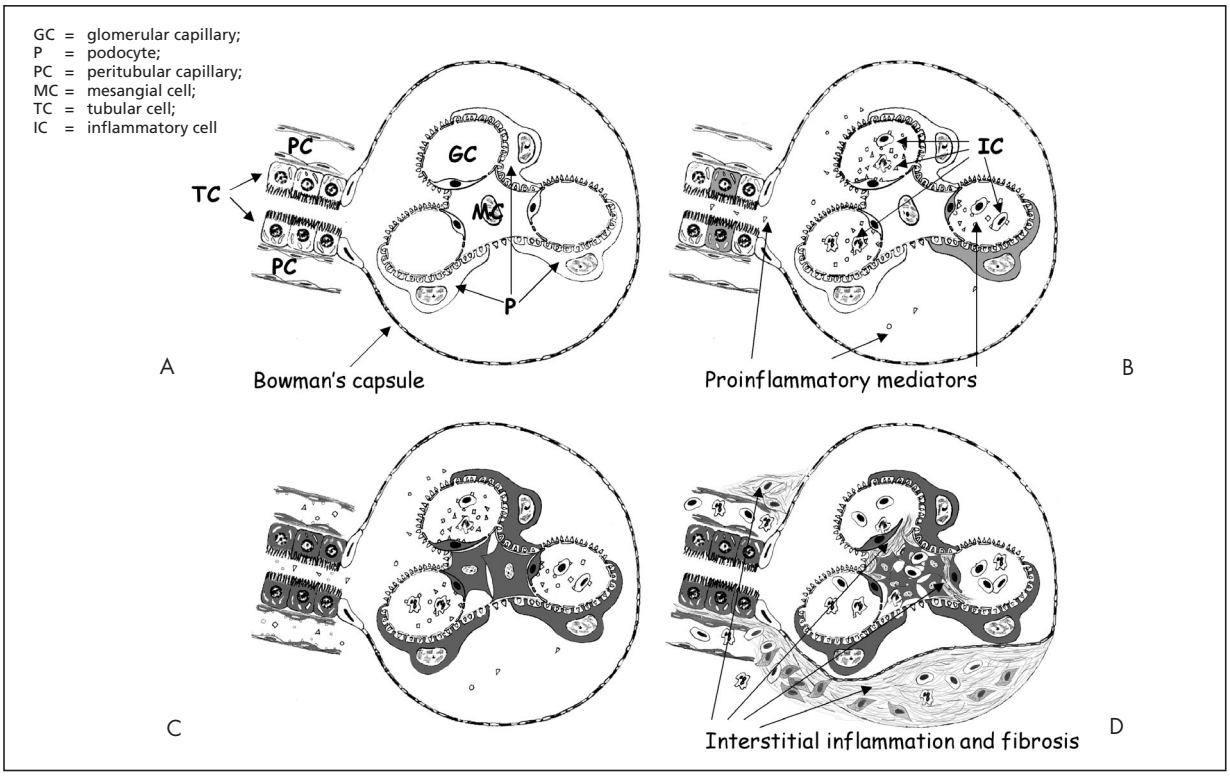

Figure 1. Potential role for chemokines in renal injury. ***A,*** Normal tissue. ***B,*** A pathologic insult to the glomerular or tubulointerstitial compartment activates intrinsic renal cells and leads to the local generation of proinflammatory mediators. This insult may target a specific renal cell type and the expression of chemokines, together with the local release of inflammatory mediators, promotes the activation of selectins and integrins on leukocites and endothelial cells leading to adhesion and infiltration of specific subsets of leukocytes (initiation phase). ***C,*** In this phase, spill of proinflammatory factors from affected glomeruli could reach the peritubular capillary circulation: protein and lipids escaping through damaged glomeruli could stress proximal tubular cells. The consequent activation of tubular and interstitial cells, in concert with the production of additional chemokines, lead to interstitial mononuclear cell infiltration. Parietal cells of Bowman's capsule could become activated and thus release chemokines into the interstitium (amplification phase). ***D,*** The periglomerular infiltrate may take part in the Bowman's capsule rupture: the consequent crossing of T cells, macrophages and fibroblasts into the urinary space lead to tissue damage (interstitial inflammation and fibrosis) (progression phase). From Segerer S. et al., J Am Soc Nephrol, 2000;11:152-176, modified.

so enrolled 30 SLE patients with a history of severe lupus nephritis but lacked current systemic disease activity after treatment (designated the disease remission group), and another 27 SLE patients who lacked systemic disease activity and had no history of renal disease (designated as nonrenal SLE group). Were further enrolled 10 healthy volunteer subjects as controls. Expression of TGF-β and MCP-1 mRNA in the urinary sediment was significantly elevated in the active disease group. These expression levels correlated with the SLEDAI score, and also significantly correlated with the histologic activity index. The urinary protein concentration of MCP-1, but not of TGF-β, correlated with the SLEDAI score. However, neither the protein concentration of TGF-ß nor that of MCP-1 as measured by ELISA in the urine correlated with the histologic activity index. In conclusion, the measurement of urinary mRNA expression may be a noninvasive method for the assessment of lupus disease activity and the severity of renal involvement in patients with lupus nephritis.

In lupus nephritis, therefore, there is local production of various inflammatory mediators, which causes infiltration of monocytes, macrophages, and T lymphocytes followed by intrarenal inflammation[44]. It has been observed a correlation between the urinary mRNA expression levels of TGF-β and MCP-1 and lupus disease activity. The mRNA levels of TGF-β and MCP-1 were significantly elevated in patients with active lupus nephritis. The gene expression levels of TGF-β and MCP-1 also correlated with

the degree of proteinuria and other important parameters related to renal impairment. Most importantly, the mRNA expression of TGF-β and MCP-1 in urinary sediment significantly correlated with the histologic activity index as scored on kidney biopsy sections. TGF-β and MCP-1 expression levels correlated with the histologic activity index, but not with the chronicity index or the degree of renal scarring as determined by formal morphometry, suggesting that the 2 cytokines are more involved in the acute inflammatory process than in chronic damage or fibrosis. The Authors observed a high degree of internal correlation between the mRNA levels of TGF-β and those of MCP-1 in urinary sediment. This result was expected, because it has been reported that MCP-1 has a profibrogenic effect through direct stimulation of TGF-β synthesis from renal cells[44], and this cytokine may stimulate the production of MCP-1 from tubular epithelial cells[45]. These data demonstrated that the mRNA expression levels of TGF-β and MCP-1 in the urinary sediment of patients with active lupus nephritis are elevated, and the degree of elevation is related to the lupus clinical disease activity and histologic activity indexes. Therefore, the measurement of mRNA by realtime quantitative PCR may be a useful noninvasive tool for clinical study of the disease activity of lupus nephritis.

Brad et al. have investigated urine MCP-1 and IL-8 as possible biomarkers of SLE flare. Urine was collected every 2 months from patients who were followed prospectively in the study[46]. Renal and nonrenal flares were identified and MCP-1 and IL-8 were measured by specific ELISA in samples that were collected at flare. When available, MCP-1 and IL-8 were also measured in urine samples before and after flare. Most patients were receiving maintenance immunosuppressive therapy before flare. At renal flare, mean urine MCP-1 was significantly greater than this chemokine at nonrenal flare and from healthy volunteers and renal disease controls. The level of urine MCP-1 correlated with the increase in proteinuria at flare and was higher in patients with proliferative glomerulonephritis and in patients with impaired renal function. Urine MCP-1 was increased beginning 2 to 4 months before flare. Patients who responded to therapy showed a slow decline in urine MCP-1 over several months, whereas non-responders had persistently high levels. In contrast, IL-8 did not change with disease activity and was not elevated at renal or nonrenal flare compared with disease controls. In conclusion, urine MCP-1 but not IL-8 is a sensitive and specific biomarker of renal SLE flare and its severity, even in patients who receive significant immunosuppressive therapy. Persistently elevated urinary MCP-1 after treatment may indicate ongoing kidney injury that may adversely affect renal prognosis.

Discussion

The infiltration of macrophages and T lymphocytes into the renal tissue have been strongly implicated in the pathogenesis of lupus nephritis[44]. Several studies have reported significant increases in the numbers of mononuclear cells in the glomerulus and tubulointerstitium of renal biopsy tissue from patients with lupus nephritis[47,48]. Intrarenal formation of inflammatory mediators, including chemokines and growth factors, has been shown to play crucial roles in the initiation and amplification of the renal inflammatory process of lupus nephritis[44,49]. Many studies have examined the concentration of inflammatory mediators at the protein level in urine and plasma, and tried to explore their possible diagnostic or prognostic value[50,51]. Kidney biopsy represent the standard method to classify the histologic condition and quantify the disease activity in patients with lupus nephritis, but it is an invasive procedure with potential complications.

Similar to allograft rejection[52], lupus nephritis is characterized by infiltration and activation of intrarenal inflammatory cells[48,53]. Since most of the inflammatory mediators exert their effects locally, measurement of their gene expression levels at the site of pathology would be a physiologically relevant approach to study the disease process.

It has been well established that proinflammatory chemokines play a crucial role in the pathogenesis of experimental SLE nephritis[54-56] and that the presence of chemokines in the urine of SLE patients with nephritis reflects intrarenal chemokine expression[35,57]. To characterize better the relationship of chemokines to SLE reactivation and to investigate the possibility that urine chemokines may be useful for noninvasively monitoring disease activity and response to therapy, some Authors obtained serial measurements of urine chemokines in a cohort of prospectively followed SLE patients and documented that urine MCP-1, but not IL-8, represent a specific bio-

marker of renal flare and the severity of kidney injury[46]. In fact: (A) urine MCP-1 increased significantly at renal reactivation compared with a control group matched for disease and medication use, and the changes in urine MCP-1 over time paralleled the onset and resolution of flare. (B) Non-renal SLE flares were not accompanied by increases in urine MCP-1 or IL-8, indicating that urine chemokines do not reflect generalized systemic SLE activity. (C) High levels of urine MCP-1 at reactivation were significantly associated with manifestations of severe renal injury, including abnormal renal function, proliferative glomerulonephritis, and the degree of proteinuria.

Urine MCP-1 increased significantly at renal flare despite moderate to intense inimunosuppression (prednisone, mycophenolate mofetil, azathioprine, or cyclophosphamide) before chemokine measurement, suggesting that MCP-1 is an important marker of SLE activity that is valid in patients who have chronic disease and are on longterm therapy. However, theese Authors emphasize that this background of immunosuppression may partially account for the fact that urine IL-8 did not significantly increase at flare, in contrast to other investigations that measured IL-8 in untreated SLE patients[58-60].

It has been documented that urine MCP-1 determination after flare demonstrated a close relationship between MCP-1 level and response to therapy. According to previous investigations[39,50], it has been well demonstrated that MCP-1 decreased in patients who responded to treatment. However, serial measurements of MCP-1 showed that it did not normalize rapidly after treatment but declined over 4 or more months, paralleling the improvement in proteinuria or creatinine. On the contrary, urine MCP-1 remained elevated in patients who did not respond to therapy.

Therefore, serial measurement of urine MCP 1 shows that it is a sensitive biomarker of renal but not nonrenal SLE, even in chronic patients who are on maintenance immunosuppressive therapy. Urine MCP-1 levels seem to predict impending flare, flare severity, and response to treatment but may be less useful for predicting renal histology. Unlike most conventional clinical biomarkers, MCP-1 is also a potential therapeutic target in SLE nephritis.

The study of Wing-Yan Chan and co-workers also demonstrated that the mRNA expression levels of TGF-β and MCP-1 in the urinary sediment of patients with active lupus nephritis were elevated, and the degree of elevation was related to the lupus clinical disease activity and histologic activity indexes[40]. Although the urinary protein levels of MCP-1 showed a trend similar to that of the mRNA expression levels of MCP-1, the difference between groups was less discriminative, especially between the remission group and the non-renal SLE group, and the protein level did not correlate with the histologic activity index. Moreover, no correlation between the urinary TGF-β protein level and biochemical or histologic parameters was found.

Preliminary results of our group confirm the lack of correlation between the urinary TGF-β protein level and biochemical and histologic findings.

In conclusion, the measurement of urinary chemokines may be a noninvasive method for the assessment of the severity of lupus nephritis, even if further studies are needed to strongly evaluate the real role of these chemokines for clinical study of the disease activity in SLE patients.

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