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PROTEINS, GROWTH FACTORS, AND PROGRESSION OF KIDNEY DISEASE

THE ROLE OF TUBULAR CELLS IN THE PROGRESSION OF RENAL DAMAGE: GUILTY OR INNOCENT?

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INTRODUCTION

The progressive development of sclerosis in remnant glomeruli after the initial renal damage is caused by hyperfiltration. Thus, there is a progressive decrease in the number of glomeruli connected to tubules. Hyperfiltration causes both proteinuria and the formation of protein casts associated with tubular atrophy. Persisting peritubular inflammation induces the release of inflammatory mediators that participate in the progression of the interstitial fibrosis. In this scenario what is the role of tubular cells?

In this review the role of proximal tubular cells will be described by reporting the "*in vitro*" and "*in vivo*" studies carried out in animal models and in humans. In addition, the therapeutic effects of drugs will be discussed.

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RENAL TUBULAR EPITHELIAL CELLS

The study of renal tubule function is complicated by the complexity and the heterogeneity of the 15 different types of epithelial cells which are present along the nephron, each exhibiting distinct morphological, biochemical and functional characteristics. During the last few years several techniques have been designed to study specific populations of renal cells. Recently, Baer et al. have described an easy and reliable magnetic cell sorting technique to separate pure populations of epithelial cells from human kidney as shown by monitoring of the pattern of surface antigens (1). Monoclonal antibody against aminopeptidase M was used for isolating proximal tubular epithelial cells (PTECs), whereas a monoclonal antibody against Tamm-Horsfall glycoprotein was used to distinguish tubular cells of the thick ascending limb and the early distal convoluted tubule.

Proteinuria remains the most important stimulus involving inflammatory genes in tubular cells thus inducing growth factors and chemokines synthesis and production (Figure 1). In addition, after reabsorption urinary proteins are processed by PTECs and their fragments are presented by the major histocompatibility complex (MHC) class II, as peptides, to interstitial inflammatory cells since MHC is not restricted to professional antigen-presenting cells, but is also present in parenchymal cells, including PTEC. This expression is very low in normal kidney whereas it is notably increased in inflammatory conditions such as tubulointerstitial damage occurring in chronic renal diseases and in acute graft rejection. Thus, PTECs by processing and presenting antigen participate in the progression of renal damage at tubulointerstitial level. PTECs might function as accessory cells for T cell activation and might support T cell dependent immune response (2).

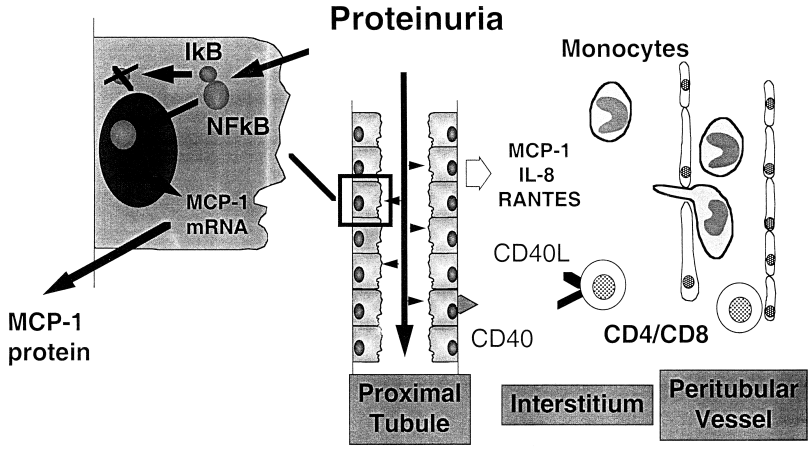

Figure 1. Chronic glomerular disease. Proteinuria is the most important stimulus which involves inflammatory genes in tubular cells.

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Interferon-gamma (IFN-γ) induces the MHC class II antigen expression on the basolateral side of PTECs, which present antigen to CD4+ positive T cells. It is well known that PTECs lack the important co-stimulatory molecules to T cells. Therefore, they deliver an anergic stimulus in many circumstances. The presence of ICAM-1 and VCAM-1 and the absence of B7 on PTEC are not sufficient to explain the reduction of activating or anergizing immune responses by PTEC. Fan et al. demonstrated, by immunofluorescence staining, the presence of a 210-κD antigen on the apical side of Henle's loop, the distal tubule and convoluted tubule (3). Recently, Banu and Meyers described the influence of TGF-β1 on class II MHC and B7-1 expression in renal tubular epithelial cells. This growth factor mediates down regulation of induced class II MHC and B7-1 expression in cultured murine renal tubular epithelial cells and does not alter induced ICAM-1 expression (4). These data suggest a significant immunomodulatory effect of TGF-β1 on renal tubular epithelial cells, in inhibiting induced B7-1 and abrogating stimulation of nephritogenic CD4+ T cells in vitro. Since the amplification process of the inflammation is represented by the activation of T and B cells and increased production of antibodies by B cells, TGF-β1 may underlie immunosuppressive effects.

Expression of interleukin-2 receptor (IL-2R) is not restricted to lymphoid cells. It has been found on human endothelial cells and human fibroblasts. Gerritsma et al. demonstrated that IL-2R, which is expressed in many pathological conditions in PTEC (5–7), may regulate the peritubular inflammatory process by modulating the local production of other cytokines, growth factors and complement components (8–10).

Proteinuria is implicated in the pathogenesis of progressive renal scarring, particularly in the tubulointerstitial compartment, since it is a prominent stimulus for chemokine production. Wang et al. demonstrated that bovine serum albumin (BSA), delipidated BSA, holotransferrin and apotransferrin induced MCP-1 mRNA expression and protein synthesis in vitro cultured PTEC. Interestingly, the concentration of this chemokine in response to delipidated BSA added to the apical surface of PTEC was 2.4 fold greater in basolateral than in apical media (11). This evidence suggests that proteinuria may stimulate PTEC production of MCP-1 and other phlogistic mediators which diffuse in the tubulointerstitial area by mediating the infiltration of inflammatory cells. The important role of MCP-1 is shown by its increased expression in a number of renal diseases and animal models, including glomerulonephritis, diabetic nephropathy, lupus nephritis, hydronephrosis and ischemia (12–16). Burton et al. showed that the stimulation of the apical side of cultured polarized PTEC by using fetal human serum and serum fractions, induces an increased basolateral release of PDGF-AB and MCP-1 in the medium. This increase was 1.7-fold for PDGF-AB and 2.4-fold for MCP-1. Fractionation of the serum showed that this effect was more prominent if induced by a fraction of molecular weight between 40 and

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100 kDa. The predominant proteins in this fraction were albumin and transferrin (17). Basolateral fibronectin production by PTECs was increased by apical exposure to serum proteins of a molecular weight 40–100 kDa, thus demonstrating the ability of PTEC to contribute to the scarring process in renal tubulointerstitium (18).

An increased synthesis of fibronectin by human PTECs in response to transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) was also demonstrated in vitro by Bürger et al. (19).

Considering that chemokine and cytokine secretion is also present at the apical level, many investigators studied the urinary levels in patients with different forms of renal diseases and correlated it with their expression in renal tissue (20–25).

PERSONAL CONTRIBUTIONS

Our group has been actively involved in the study of the local mechanisms implicated in the progression of renal damage in patients with different forms of glomerulonephritis. Recent data from our laboratory and from other groups in the attempt to evaluate their relative impact on the clinical course of the disease will be reviewed.

The kidney is one of the most important sites of production of epidermal growth factor (EGF) which seems to play several biological functions such as the modulation of tubular cell growth, renal repair at tubular level after acute injury and regulation of cellular metabolism. This growth factor is usually expressed and synthesized by tubular cells in Henle's loop and distal convoluted tubule. We observed a drastic decrease of EGF mRNA expression and protein synthesis in patients with IgA nephropathy (IgAN) and severe tubulointerstitial damage (26,27). The EGF renal expression was strictly correlated with grade of tubular lesions and the urinary excretion, which reduced drastically in patients with severe renal damage (27).

Monocyte-chemoattract protein-1 (MCP-1) is produced by a variety of cells including tubular epithelial cells and macrophages. This chemokine binds cells with a specific receptor as CCR2. MCP-1 is a powerful and specific chemotactic factor for monocytes and in addition it activates these cells inducing calcium influx, respiratory burst, adhesion molecule expression and cytokine production (28). A striking increase in MCP-1 gene expression and protein synthesis was present at tubulointerstitial level in IgAN patients with severe renal damage. MCP-1 gene expression correlated with the extension of interstitial monocyte infiltrate, depicted by CD68+ cells, tubular atrophy and interstitial fibrosis. Urine excretion of MCP-1 was notably increased in patients with severe renal damage. Recently we measured the EGF/MCP-1 ratio in the urine of IgAN patients who were enrolled for a randomized clinical trial (unpublished data). Patients were divided in three

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groups: (a) subjects with normal renal function (serum creatinine < 1.5 mg/dL and proteinuria < 1.0 g/day); (b) patients with normal renal function and proteinuria > 1.0 g/day; (c) patients with chronic renal insufficiency and moderate proteinuria (> 1.0 g/day). At the beginning of the study patients included in group (a) had a high EGF/MCP-1 ratio; patients of group (b) had a moderate reduction of the ratio and patients of group (c) showed very low EGF/MCP-1 ratio. After two years of treatment with ramipril IgAN patients with moderate renal damage showed a slight improvement of the urinary EGF/MCP-1 ratio which increased significantly in those treated with the association of ramipril and trapidil (anti-platelet drug which blocks the PDGF receptor). These data suggest that the urinary EGF/MCP-1 ratio may be a reliable marker of disease activity, which may be used for the monitoring of the therapy efficacy.

Chronic obstructive nephropathy is characterized by tubulointerstitial damage such as tubular atrophy, monocyte infiltration and interstitial fibrosis. Although the acute hemodynamic and functional abnormalities associated with obstructive uropathy have been clarified, the cellular and molecular mechanisms leading to the chronic renal lesions are still largely unknown. We studied the EGF and MCP-1 mRNA expression in renal biopsies performed in patients with congenital ureteropelvic junction obstructive uropathy (29). However, at the time of the study some of the patients had clinical signs or laboratory markers of active urinary infection. Some of them had recurrent urinary tract infections. Renal samples were obtained at the time of surgical treatment (pieloplasty). Tubular damage, mainly characterized by tubular dilatation and tubular cell atrophy, was the characteristic hallmark of the obstructive uropathy. The renal EGF mRNA expression was strikingly reduced within the Henle's loop and distal tubules. This decrease was directly correlated with the degree of tubular damage. The reduced EGF mRNA expression paralleled with its urine excretion, which was significantly low.

The interstitial inflammation is a constant feature of the chronic obstructive uropathy. The infiltrate is constituted by monocytes and T lymphocytes. We found an increased MCP-1 mRNA expression into the interstitium within the tubules, the infiltrating mononuclear interstitial cells and the endothelial cells of small vessels. It was directly correlated with the extent of monocyte infiltration. A great amount of CD68+ cells was present in the infiltrate strongly supporting the role of MCP-1 as chemokine for the recruitment of interstitial monocytes. The high presence of MCP-1 in renal tissue mirrored its urinary excretion, which increased mainly in patients with associated recurrent urinary tract infections. Interestingly, urinary MCP-1 levels correlated significantly with the mean time of transit indicated by the 99TC-MAG3 scan through the cortex of the obstructed kidney. In addition, the MCP-1 urine concentration collected directly from the obstructed pelvis at the time of the surgical treatment was inversely correlated with MAG 3

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clearance of the diseased kidney. This means that there is a link between the inflammatory response and the tubular function and the possibility of a direct involvement in the progression of renal damage in obstructive nephropathy. After surgical treatment, MAG 3 clearance improved drastically in patients with previous obstruction and not in those with no obstruction. We measured the urinary concentration of EGF and MCP-1 in these patients and an improvement of EGF urine excretion and a reduction of MCP-1 urine excretion was observed after pyeloplasty. In addition, the EGF/MCP-1 urinary ratio improved in patients after the surgical correction of the congenital ureteropelvic junction obstruction. These data suggest that EGF/MCP-1 ratio might be a valuable prognostic marker for the progression of renal damage and may help physicians in monitoring the clinical course of the disease after therapy.

CONCLUSIONS

Renal injury is characterized by a decreased nephron mass, glomerular hyperfiltration and proteinuria, which permanently stimulates tubular cells in the production of cytokines, growth factors and chemokines. These inflammatory mediators contribute to the progression of renal damage characterized by monocyte infiltration and extracellular matrix deposition. Proteinuria stimulates basolateral production of the inflammatory mediators, which diffuse in the peritubular area. However, a large number of these mediators are present in the urine. We reviewed our data on the mRNA expression of EGF and MCP-1 in renal biopsy performed in patients with different renal disease and we also measured the urinary concentration of these phlogistic mediators. Interestingly, we found that urinary concentration correlated with their expression at renal level and that the urinary EGF/MCP-1 ratio was a valuable marker for the monitoring of the disease during and after therapy. These data suggest that molecular biology applied to renal biopsy may help in searching for urinary markers useful to monitor the progression of renal damage in patient with chronic nephropathies.

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