Pathophysiology of Tubulointerstitial Disease

Morphometry of interstitial fibrosis

Emile De Heer¹, Yvo W. J. Sijpkens², Martijn Verkade¹, Marcel den Dulk², Alexandra Langers², Jan Schuttrups³, Jan Anthonie Bruijn¹ and Leendert A. van Es²

Departments of ¹Pathology and ²Nephrology, Leiden University Medical Centre, Leiden, The Netherlands

Abstract Several clinical studies have confirmed that histomorphometric changes in the tubulointerstitial compartment contain the best correlating parameters to predict the development of progressive renal insufficiency. The process of interstitial fibrosis is accompanied by an influx of inflammatory cells, up-regulation of fibrogenic cytokines such as transforming growth factor-β and basic fibroblast growth factor, transient down-modulation of their antagonists, generation and proliferation of myofibroblasts, and, finally, by accumulation of interstitial collagens and proteoglycans. A careful morphometric analysis of interstitial fibrosis requires sensitive parameters through which the severity can be quantified and by which the progression into renal insufficiency can be predicted. We have addressed these issues by morphometric analysis of both human biopsies and by refining existing experimental models in the rat. Morphometric analysis was performed using a Zeiss microscope equipped with a full colour 3CCD camera and KS-400 image analysis software from Zeiss-Kontron. For studies with human material, biopsies were examined from patients with various renal diseases including patients with chronic allotransplant dysfunction. The development of interstitial fibrosis was correlated with clinical parameters. In experimental models, we analysed the interstitial composition and eventual glomerular alterations in rats with bovine serum albumin (BSA)-induced protein overload nephropathy and with human IgG-induced chronic serum sickness nephritis. Finally, we adapted and refined the model of ureter obstruction-induced interstitial fibrosis in the rat. For this purpose, custom-made titanium clips (S&T, Neuhaus, Switzerland) were implanted around the ureter in the abdomen of rats to obstruct the ureter without causing necrosis. The clips were removed at various time points after obstruction of the ureter (1–14 days). The subsequent remodelling of the interstitium was studied thereafter, in order to establish whether uremia-induced interstitial fibrosis remains reversible at all times. In rat models, we have found that both protein overload-induced and serum sickness-induced interstitial fibrosis are accompanied by the development of focal and segmental glomerulosclerosis. Only in the ureter obstruction model did selective interstitial fibrosis develop, and remained reversible at all times studied. For the reliable assessment of interstitial fibrosis we have found that the best correlating parameters of interstitial fibrosis with renal function were: (i) the ratio of protein accumulation of TGF-β-1 and its antagonist decorin; (ii) interstitial expression of smooth muscle α-actin; and (iii) accumulation of interstitial collagens (as determined by immunoperoxidase and by Sirius red staining).

Introduction

Interstitial changes leading to progressive renal insufficiency can occur after a variety of injury pathways including chronic allograft rejection, hypertension, proteinuria and chronic inflammation [1]. For the development of interstitial fibrosis, several inducing factors have been identified, of which transforming growth factor-β1 (TGF-β1), endothelin-1, matrix metalloprotease-2 (MMP-2), angiotensin II, osteopontin and vascular endothelial growth factor (VEGF) are the most important. The concerted action of these factors results in an increased influx of alpha smooth muscle actin-positive myofibroblasts, increased local transcription of EDA-positive, MMP-2-resistant fibronectin, accumulation of interstitial collagens and selective degradation and down-modulation of decorin and MMP-9 [2–6].

We have analysed several experimental models for interstitial fibrosis in order to establish the optimal experimental model to study both the induction and the remodelling phase of tubulointerstitial injury. The expression of several ‘effectors’ was investigated morphometrically by computer-assisted image analysis. General precautions for adequate morphometry of the tubulointerstitium for reproducible measurements have to be made. First, all tissue sections that are stained with one antibody have to be incubated and developed in one session simultaneously. Second, the glomeruli, the peri-glomerular area (a ring of one tubular diameter in size) and large blood vessels have to be excluded.
from the morphometric analysis. Third, both the microscope, the CCD camera, the frame grabber, the light source (>15 min warm-up time) and the software (shading correction, definition of thresholds) have to be calibrated accurately. When automated repetitive microscopic fields within one section are measured, a motor-driven autofocus is necessary.

Experimental results

Rats with bovine serum albumin (BSA)-induced protein overload nephropathy [7,8] developed proteinuria and only mild interstitial fibrosis. Long-term experiments (>20 weeks) resulted in the development of focal and segmental glomerulosclerosis. Unilateral ureter obstruction [9] resulted in an extensive infiltration of the tubulointerstitium by T cells, macrophages and myofibroblasts. Subsequently, severe interstitial fibrosis developed, as detected by Sirius red histochrometry [10], without any detectable glomerular abnormalities. Restoration of the urinary flow by removal of the obstruction clips up to 14 days after ureter obstruction resulted in total remodelling of the interstitium. After 14 days of obstruction, urinary flow could no longer be restored because of irreversible fibrotic adhesion of the ureter.

Results from human biopsies

In order to investigate whether the composition of the interstitial infiltrate correlates with either chronic rejection or with chronic cyclosporin toxicity, we analysed 22 biopsies from patients who lost their graft due to chronic allograft rejection [11], and 14 biopsies from patients with clinically and histologically defined (arteriolar hyalinosis) chronic cyclosporine toxicity [12]. Tissue sections were stained with markers for inflammatory cells (CD45), T lymphocytes (CD45RO and CD45RA), macrophages (CD68) and smooth muscle α-actin (SMA). The area percentage of the entire cortex (magnification × 100) was measured by digital image analysis. Grafs with chronic rejection showed significantly more infiltration of CD45- and CD45RO-positive (memory) T cells. However, significantly more smooth muscle actin expression was observed in grafts with cyclosporin toxicity.

A second analysis was performed to analyse whether protein expression of the fibrogenic cytokine TGF-β1 and its natural antagonist decorin [2,14] would correlate with the progression of interstitial fibrosis. For this purpose, in 54 human biopsies from patients with various renal diseases [minimal change nephropathy (MCD), lupus, IgA nephropathy, FSGS] and in five normal controls, the protein expression of TGF-β1 and its natural antagonist decorin was measured by morphometry. In all samples with interstitial fibrosis, an up-regulation of TGF-β1 was observed in combination with a reduced expression of decorin. This was not observed in 18 biopsies from patients with MCD, in which interstitial fibrosis does not develop. A high decorin/TGF protein expression ratio correlated with high proteinuria levels. This analysis shows that dysregulation of TGF-β1 and decorin [13] in either direction leads to reduction of renal function.

References