Immunohistochemical Localization of Laminin in Renal Lesions of Balkan Nephropathy

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Dear Sir

Balkan nephropathy is a chronic tubulointerstitial kidney disease of unknown etiology [1]. Kidney morphology in early stages of the disease is peculiar and resembles that of ageing with pronounced renal vascular changes. Tubular atrophy and interstitial sclerosis are predominant in advanced stages [2-4]. The distribution of the extracellular matrix components has been studied previously in human glomerular diseases in order to elucidate their pathogenesis, and especially that of glomerulosclerosis [5, 6]. In the present study, we have investigated the immunolocalization of laminin, the major non-collagenous basement membrane protein, in the kidney of patients with different stages of Balkan nephropathy.

This study included 12 renal biopsies from patients with Balkan nephropathy diagnosed according to the criteria previously described [1]. The mean age of examinees was 53.8 years (range 49-58). All but 3 exhibited renal failure, with a mean serum creatinine of $113 \mu\text{mol/l}$ (range 75-142), and $^{99\text{m}}\text{Tc}$ -DTPA clearance of 74.3 ml/min (range 28-122). Arterial blood pressure was normal or moderately increased (mean $138.3/90.8 \text{ mm Hg}$). Ten kidney specimens of apparently healthy accident victims, obtained at autopsy 6-10 h after death, served as controls. For the histologic analysis, biopsy specimens were fixed in alcoholic Bouin's fixative for 12-24 h followed by routine processing and embedding in paraffin. Frozen sections were first incubated with the laminin antibody. Subsequently, avidin-biotin-complexed specific antirabbit antibody (Sigma Chemical Co., St. Louis, Mo., USA) was used as secondary antibody. The avidin-biotin complexes of the secondary antibodies were visualized with aminoethylcarbazole, and sections were evaluated by routine light microscopy.

In normal kidneys, laminin was present in the mesangium, glomerular basement membrane, tubular basement membrane and Bowman's capsule. The interstitium was negative for laminin. Our results are in agreement with the quantitative immunocytochemistry data of Desjardens and Ben-dayan [7], who demonstrated labeling for laminin over all basement membranes. For this antigen, the proximal tubular basement membrane was the most intensely labeled. The mesangial matrix and the glomerular and distal tubular basement membranes displayed intermediate density, whereas that of Bowman's capsule was significantly lower. Few gold particles were observed over the cell cytoplasm and capillary lumina [7].

In the early stage of Balkan nephropathy, a marked overexpression of laminin in renal interstitial capillaries was observed with a moderately increased expression in tubules (fig. 1a). Later stages were characterized by the intensive expression of laminin in atrophic tubules, much more in proximal than in distal ones. Increased expression of laminin in glomerular capillaries was also demonstrated, sometimes it was segmental (fig. 1b). The pattern of laminin staining in glomeruli corresponded to focal and segmental glomerular sclerosis present in the advanced stages of Balkan nephropathy.

This study confirms that major changes in Balkan nephropathy are localized in interstitial capillaries, occurring in the early stages. Markedly increased laminin expression in interstitial capillaries was demonstrated in patients with normal blood pressure. Marked tubular atrophy, especially in proximal tubules, was associated with an overexpression of laminin. The observed changes were much pronounced in the outer cortex.

The extracellular matrix acts not only to support glomerular/tubular cells but also conveys information to them and modifies their behavior [8]. Little is known about the role of extracellular matrix proteins in glomerular disease, and this study gives an account of renal distribution of laminin in Balkan nephropathy. Evidence is presented confirming that renal vascular changes occur early in Balkan nephropathy.

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Fig. 1. Distribution of laminin in renal sections. a Early-stage kidney with marked expression of laminin in vascular structures. b Increased staining for laminin in basement membranes of atrophic tubules and glomerular capillaries.

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