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Biochemical studies on the effect of advanced glycation end products on renal tubular epithelial cells in diabetic nephropathy

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We studied the activation of nuclear factor kappa B (NF-κB) in human proximal tubular epithelial cells (pTEC) by advanced glycation end products (AGE-albumin) and carboxymethyllysine (CML) in vivo and in vitro, since previous studies have shown that renal function in Type 2 diabetes correlates better with tubular changes than with glomerular pathology.

Urine samples from type 2 diabetic patients were tested for excretion of proximal tubular epithelial cells (pTEC) and stained for activation of the redox-sensitive transcription factor NF-κB p65. Urine sediments of 20 out of 50 patients (40%) with overt diabetic nephropathy contained pTECs, evidenced by cytokeratin 18 positivity, while diabetic patients without diabetic nephropathy (n = 50) and healthy controls (n = 50) showed none ($p < 0{,}0001$). Activated NF-κB could be detected in the nuclear region of excreted pTEC in 8 out of 20 patients with pTEC in the urine sediment (40%). Five of 8 NF-κB p65-antigen positive cells stained positive for interleukin-6 (IL-6) antigen (62%), while only one of

the NF-κB negative cells showed IL-6 positivity. In addition, in type 2 diabetic patients with overt diabetic nephropathy, we observed a positive correlation between the amount of urinary excreted CML and the degree of albuminuria ($r = 0.4$, $p = 0.002$). Immunohistochemistry confirmed a strong expression of NF-κB in tubular cells in a human diabetic kidney and in kidneys from diabetic rats.

To study the mechanism, NF-κB activation was studied in cultured human pTEC by electrophoretic mobility shift essays (EMSA) and Western-Blot. Stimulation of NF-κB binding activity was dose-dependent, being 1/2 maximal at 250 nM AGE-albumin or CML and time-dependent with a maximum of activation after 4 days. Functional relevance of the observed NF-κB activation was demonstrated in pTEC transfected with a NF-κB driven luciferase reporter plasmid and was associated with an increased release of interleukin-6 (IL-6) into the supernatant. The AGE and CML-dependent activation of NF-κBp65 could be inhibited using the soluble form of the AGE-receptor RAGE or a RAGE-specific antibody and by the antioxidant thioctic acid. Taken together, our data underline the role of pTEC as primary targets in diabetic nephropathy.