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Proteoglycans can mediate renal lipoprotein retention

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To the Editor:

Diabetes is the leading cause of end-stage renal disease in the US. It is increasingly accepted that hyperlipidaemia aggravates the progression of renal disease. Glomerular capillaries are separated from the mesangium by a fenestrated endothelium without a basement membrane. Thus macromolecules such as lipoproteins have relatively free access to the mesangium. Increased renal deposition of apolipoproteins (apo) B and/or E is associated with increased mesangial cellularity, increased proteinuria, and increased severity of glomerulosclerosis in a variety of glomerular diseases in humans [1]. The mechanism leading to mesangial lipoprotein accumulation is unknown. Proteoglycans are a heterogeneous group of molecules composed of a core protein to which one or more glycosaminoglycan side-chains are attached. The expression of renal proteoglycans is altered by development of renal disease in humans [2], and altered proteoglycan expression correlates with loss of renal function. The aim of this study was to determine if mesangial proteoglycans would bind LDL, as a mechanism contributing to renal lipoprotein accumulation.

Rat mesangial cells (American Type Culture Collection, Manassas, VA, USA) were grown to confluence in RPMI medium supplemented with 10% fetal bovine serum, pyruvate, non-essential amino acids, 4 mmol/l glutamine and antibiotics, then made quiescent for 48 h by lowering the serum concentration to 1%. Cells were metabolically labelled with 3.7 MBq/ml [³⁵S]sulphate or 1.85 MBq/ml [³⁵S]methionine, then secreted proteoglycans were collected as previously described [3]. Proteoglycans secreted by mesangial cells were incubated with native human LDL for 1 h under physiological conditions of pH,

temperature and ionic strength, and subjected to gel mobility shift assays [3]. Analyses of the binding kinetics (using GraphPad Prism Software) demonstrated that mesangial proteoglycans have high binding affinity for LDL ($K_d=14\pm5$ μ g/ml LDL; mean $\pm$ SEM, $n=3$; Fig. 1). Since TGF- β is an established mediator of diabetic nephropathy [4], the effect of TGF- β on the LDL binding affinity of mesangial proteoglycans was determined. Proteoglycans synthesised by mesangial cells stimulated with TGF- β (2 ng/ml for 24 h) demonstrated increased LDL binding affinity ($K_d=3.7\pm1.2$ μ g/ml LDL; mean $\pm$ SEM, $n=3$) compared with proteoglycans synthesised by unstimulated cells ($p<0.01$, Fig. 1). The binding affinity of proteoglycans for LDL is thought to be determined by the length and degree of sulphation of their glycosaminoglycan chains [3, 5]. Characterisation of the proteoglycans secreted by mesangial cells stimulated with TGF- β confirms previous observations showing increased [³⁵S]sulphate incorporation and increased size compared with proteoglycans synthesised by unstimulated cells [6]. Thus, we report that TGF- β has similar effects on mesangial and vascular proteoglycan synthesis [5]. Conversely, the thiazolidinediones troglitazone or rosiglitazone did not affect the LDL binding affinity of unstimulated or TGF- β -stimulated mesangial proteoglycans (data not shown), thus suggesting that proteoglycans from different tissues are affected differently by these agents [3].

Considerable evidence suggests that renal accumulation of lipoproteins is toxic. Increased renal apo B and E staining is associated with more advanced renal disease [1]. LDL has been shown to promote mesangial cell proliferation, to enhance the secretion of TGF- β , platelet-derived growth factor, IL-6 and macrophage colony-stimulating factor, and to increase the synthesis of matrix proteins, including increased synthesis of proteoglycans (reviewed in [7]). Mesangial proteoglycan synthesis is increased in diabetes [2], probably due to increased TGF- β activity in this disease [4]. We now demonstrate that mesangial proteoglycans exhibit high binding affinity for LDL. The binding constants calculated for the interaction of mesangial proteoglycans with LDL are similar to constants

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Fig. 1 Mesangial proteoglycans bind LDL with high affinity. **a** Radiolabelled proteoglycans secreted by quiescent mesangial cells were incubated with the indicated concentrations of native human LDL under physiological conditions and then analysed by gel mobility shift assays. Proteoglycans that are bound by LDL remain at or near the origin of the gel; unbound or free proteoglycans migrate into the gel. A gel representative of three independent experiments is shown. **b** Proteoglycans synthesised by mesangial cells stimulated with 2 ng/ml TGF- β 1 (*solid squares*) demonstrate higher LDL binding affinity than those synthesised by unstimulated cells (*open squares*). Binding is calculated as percentage of radioactivity at the origin relative to total radioactivity in each lane. Binding curves shown are means $\pm$ SEM of three independent experiments

calculated for vascular proteoglycans [3, 5], and are physiologically relevant given the estimated tissue concentrations of LDL [8]. The results suggest that increased TGF- β activity in diabetes can alter mesangial proteogly-

can synthesis leading to enhanced retention of LDL, thus contributing to the progression of renal pathology. We suggest that renal proteoglycans be evaluated as a potential target of intervention in the treatment and/or prevention of diabetic nephropathy.

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