Diabetic nephropathy: The central role of renal proximal tubular cells in tubulointerstitial injury

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Summary. Diabetic nephropathy is now the commonest cause of end stage renal disease and accounts for 30-40% of all patients requiring renal replacement therapy. Furthermore, the incidence of diabetic nephropathy continues to increase, in part due to the improved survival of type 2 diabetic patients as the cardiovascular mortality in this group declines (Ritz and Stefanski, 1996). Clinically incipient nephropathy is first manifest by the onset of persistent microalbuminuria, after which, overt diabetic nephropathy is heralded by the appearance of persistent proteinuria. Subsequently, there is a progressive decline in glomerular filtration rate (GFR) resulting, within 5 years, in end stage renal disease in 50% of patients (Hasslacher et al., 1989). The pathology of the renal lesions are similar in type I and II diabetes (Taft et al., 1994), although it has been suggested that there is more heterogeneity in type II diabetes (Chihara et al., 1986). Studies analysing structural-functional relationships have demonstrated that the development of proteinuria correlates with the degree of mesangial expansion (Mauer et al., 1984; White and Bilous, 2000). Although diabetic nephropathy was traditionally considered a primarily glomerular disease, it is now widely accepted that the rate of deterioration of function correlates best with the degree of renal tubulointerstitial fibrosis (Mauer et al., 1984, Bohle et al., 1991). This suggests that although in the majority of patients the primary event is a condition manifest by glomerular changes resulting in proteinuria, the long-term outcome is determined by events in the renal interstitium. With the increasing awareness of the importance of these pathological interstitial changes, interest has focused on the role of cells, such as the epithelial cells of the proximal tubule (PTC) or the interstitial myofibroblast, in the initiation of fibrosis. The aim of the present review is to analyse the available data supporting the role for the PTC in orchestrating renal interstitial fibrosis in diabetic nephropathy as a result of glucose-dependent alterations in PTC function. The potential for subsequent effects on PTC-fibroblast cross-talk will also be considered.

Key words: Diabetes, Diabetic nephropathy, Interstitial fibrosis

Glucose, PTC and adaptive changes

Hyperglycaemia has been implicated as a major factor in the aetiology of diabetic nephropathy. However, the molecular mechanisms linking hyperglycaemia to the development of nephropathy are not completely understood. Structural alterations related to diabetes mellitus may be divided into early adaptive alterations and the later pathological changes associated with progression of nephropathy. In the glomerulus this is exemplified by early glomerular hypertrophy and glomerular basement membrane thickening, neither of which signify progressive renal disease, and mesangial matrix expansion and alterations in mesangial cellularity, culminating in glomerulosclerosis. Similarly in the tubulointerstitium, tubular hypertrophy and associated basement membrane alterations precede tubulointerstitial fibrosis, which accompanies progressive renal dysfunction. Recent studies have clearly demonstrated that an increase in tubular basement membrane (TBM) width is an integral component of early nephropathy (Brito et al., 1998). Furthermore alterations in TBM width were more closely correlated with the levels of glycosylated haemoglobin than other structural parameters. This therefore suggests a direct effect of hyperglycaemia on the turnover of the TBM matrix components. Studies with a murine cortical tubular cell line have demonstrated induction of collagen gene transcription and secretion in response to high glucose concentrations (Ziyadeh et al., 1990, 1991). Other studies using primary cultures of human renal proximal tubular cells have also demonstrated that exposure to 25mM D-glucose increased the amount of type IV collagen and fibronectin in the culture supernatant, which was not dependant on de novo protein synthesis (Phillips et al., 1997a-c). The accumulation of matrix
proteins is the result of a change in the balance between their synthesis and degradation and the observed increases in collagen and fibronectin were found to be associated with an alteration in their degradative pathway. Although exposure of human proximal tubular cells (HPTC) to elevated glucose concentrations increased gelatinase A enzymatic activity, as a result of induction of TIMP1 and TIMP2, there was a net decrease in gelatinolytic activity which accounted for the accumulation of fibronectin and type IV collagen. In diabetes, proximal tubular cells may be exposed to conditions of elevated glucose either apically as a result of glycosuria, or basally as a result of elevated interstitial tissue concentrations of glucose. It has been demonstrated that glucose mediated alterations in the turnover of matrix components may be stimulated by elevated concentrations of glucose at either the apical or basolateral aspect of the cells, although the alterations in matrix turnover are manifest only on their basolateral aspect (Morrissey et al., 1999a,b).

The renal reabsorption of glucose is trans-cellular. Glucose is concentrated across the luminal plasma membrane by Na+-glucose co-transporters (Na+-GLUT), which derive the energy for glucose transport by coupling glucose to the entry of Na+. Glucose efflux from tubules into the interstitium is through basolateral facilitative glucose transporters and is energised by the outwardly directed glucose gradient created by the Na+-dependent influx. In diabetic rat tubules, adaptation of GLUT1 and GLUT2 expression and activity are associated with augmented facultative glucose flux, which results in a higher intracellular glucose load (Dominguez et al., 1994). Interestingly, accumulation of glucose is not a polarised phenomenon, and occurs following application of glucose to either the apical or basolateral aspect of the cell. One consequence resultant from intracellular glucose accumulation is activation of the polyl pathway. The metabolism of hexose or pentose sugars by this pathway is of great importance in tissues which do not require insulin for glucose uptake, such as the kidney, lens, retina and peripheral nerves. Importantly these organs are the major sites of diabetic complications. Numerous groups have demonstrated the importance of this pathway in glucose mediated alterations in proximal tubular cell matrix generation (Ziyad et al., 1991; Bleyer et al., 1994; Morrissey et al., 1999a,b). It has however been demonstrated that there is a significant amount of variability in the ability of cultured proximal tubular epithelial cells (PTC) from different individuals to activate the polyl pathway (Flath et al., 1992). This therefore may be one factor, of many, which explains the heterogeneous nature of diabetic nephropathy. It is clear however that this pathway is not the sole mediator of glucose induced changes in PTC function.

In addition to alterations in TBM width, tubular hypertrophy is an early renal structural feature of diabetes. In contrast to alterations in matrix generation, cell hypertrophy is not mediated by the polyl pathway (Ziyad et al., 1991). In contrast, the hypothesis has been put forward that high concentrations of filtered glucose contribute to cell hypertrophy by increasing proximal tubular sodium absorption (Kumar et al., 1988; Bank and Aynedjian 1990), as an increase in intracellular sodium concentration has been shown to play a pivotal role in the development of cellular hypertrophy (Stanton and Kaissling, 1989). It has been demonstrated that in isolated perfused tubules glucose added to the luminal fluid, but not the abluminal fluid/bath increases fluid absorption and transepithelial electrical potential (Burg et al., 1976). In addition, in both normal and diabetic rats, progressive increases in intra-luminal D-glucose concentration stimulate net sodium and water reabsorption (Bank and Aynedjian, 1990). A rise in intracellular sodium has also been reported in vitro when proximal tubular cells were exposed to an elevated glucose concentration (Kumar et al., 1988). As diabetes is also associated with an increase in Na/K ATPase activity (Pollock et al., 1991), it is likely that the increase in intracellular sodium relates to stimulation of apical sodium entry by brush border Na-glucose co-transporter (Bank and Aynedjian, 1990) beyond the rate of basolateral exit (Pollock et al., 1991). This enhanced tubular transport of sodium, is thought to be causally related to renal/tubular hypertrophy. Although the mechanism, by which cell hypertrophy is mediated is not entirely clear, several possible mechanisms have been put forward including alteration of alteration of intracellular calcium (Haworth et al., 1980), Protein kinase C activation (Takai, 1979), alteration of Na/H exchanger activity and alteration of cell alkalisation (Fine et al., 1985).

Glucose, PTC and alterations in renal structure associated with progressive renal disease

1. Influence of glucose on PTC Hyaluronan synthesis

In addition to alterations in the turnover of “normal” matrix constituents, the diabetic state may also lead to the de novo induction of “abnormal” structural elements, such as hyaluronic acid (HA). HA is a water-soluble glycosaminoglycan, which is a key constituent of the pericellular matrix and has important structural functions in the extracellular matrix of all tissues. It has only recently been recognised that it can perform more subtle functions than just serving as a structural scaffold. HA may function as a cellular signalling molecule, following either binding to its cell surface receptors, (CD44 and RHAMM) or following internalisation via CD-44 mediated endocytosis (Hua et al., 1993). HA has therefore been implicated in a number of biological processes, including embryonic development, tumour growth, chronic inflammation and wound healing (Laurent and Fraser, 1992).

In the normal kidney HA is expressed in the interstitium of the renal papilla only, however it is known to be expressed around proximal tubular cells
following renal injury (Lewington et al., 2000), and during progressive interstitial fibrosis (Wells et al., 1990, 1993; Sibalic et al., 1997). Although alterations in HA generation have been implicated in the pathogenesis of the glomerular lesions of diabetic nephropathy (Mahadevan et al., 1995, 1996), much less is known regarding its regulation in the interstitium in the diabetic state. Recent studies using cultured human proximal tubular cells, however, have demonstrated increased HA synthesis in response to either IL-1β or elevated 25 mM D-glucose is associated with nuclear factor NF-kB activated transcription of the hyaluronan synthase gene HAS 2 (Jones et al., 2001). It has been shown that hyaluronan induces monocyte chemoattractant protein-1 expression (Beck-Schimmer et al., 1998) and up-regulation of ICAM-1 and VCAM-1 (Oertli et al., 1998) in renal tubular epithelial cells, the latter also involving activation of NF-kB. Although primarily a “metabolic” condition, macrophage influx, has been implicated in the pathogenesis of interstitial fibrosis associated with diabetic nephropathy. It is interesting to speculate therefore that the alteration in PTC HA generation may be a key factor which regulates leucocyte recruitment into the cortico-interstitium and also their interaction with resident cells in diabetic nephropathy.

2. Influence of glucose on PTC TGF-ß1 generation

Transforming growth factor β1 (TGF-ß1) has emerged as a mediator which is implicated in the pathogenesis of fibrosis in both glomerular and interstitial compartments of the kidney and has been particularly associated with diabetic nephropathy (Yamamoto et al., 1993). It is now clear that epithelial cells of the proximal tubule have the potential to contribute to the pathogenesis of renal fibrosis by the production of pro-fibrotic growth factors, including TGF-ß1 (Phillips et al., 1995, 1996, 1997a-c). Recent studies have demonstrated that TGF-ß1 production in PTC may be controlled at the levels of transcription, translation and secretion of pre-formed protein as well as at the level of activation of the latent protein (Phillips et al., 1995, 1996, 1997a-c). In the context of diabetic nephropathy the synergistic action of elevated concentrations of glucose with cytokines such as platelet derived growth factor (PDGF) or interleukin 1ß (IL-1ß) will stimulate TGF-ß1 synthesis by PTC (Phillips et al., 1995, 1996). In these studies, induction of TGF-ß1 synthesis resulted from the stimulation of translation of glucose-induced TGF-ß1 mRNA by either cytokine, and was associated with an alteration in its inherent stability. From these studies, it is interesting to speculate that although glucose primes PTC for TGF-ß1 synthesis, that glucose stimulated leucocyte recruitment via hyaluronan generation is necessary for TGF-ß1 protein synthesis. In contrast, studies utilising SV40 transformed proximal tubular epithelial cells, demonstrated that exposure to high glucose alone was sufficient to increase their generation of TGF-ß1 (Rocco et al., 1992).

Transformation by SV40 may be accompanied by alterations in response to cytokines, which may explain discrepancies in results between transformed and non-transformed cells (Wang et al., 1996). The results obtained in primary cultures of HPTC, however, are similar to those observed in studies using non-transformed endothelial cells. These demonstrated inhibition of cell proliferation and increased gene expression of basement components and TGF-ß1, on addition of high concentrations of D-glucose (Cagliero et al., 1988a,b, 1991). Significantly, however, there was no increase in active TGF-ß1 production, as assessed by bioassay. Furthermore the effects of elevated D-glucose on cell proliferation and basement membrane component gene expression, were not antagonised by the addition of neutralising antibodies to TGF-ß1 (Cagliero et al., 1995). These results therefore suggest that glucose alone may be insufficient to induce TGF-ß1. This is consistent with the clinical observation that hyperglycaemia alone is not sufficient to initiate progressive renal disease in all patients.

3. Functional role of PTC-derived TGF-ß1

(a) Effect on PTC

Many laboratories, including our own, have begun to investigate the role of the interstitial fibroblast in the progression of fibrosis. In the normal interstitium there are relatively few of these cells and they lie in close apposition to the PTC basement membrane. In progressive disease, however, their numbers increase and they take on the phenotypic appearance of activated myofibroblasts. These cells are believed to be the major source of the expanded extracellular matrix and their presence has been suggested as the best marker for progressive disease. The cellular origin of the myofibroblast, however, remains largely unknown. Hypotheses put forward include proliferation and activation of resident interstitial cells, migration of fibroblasts into the site of injury or the transdifferentiation of PTC. Of these the latter has particularly received increasing attention in recent years.

Transdifferentiation is defined as a shift in the phenotype of a differentiated cell to that of another cell type. Transdifferentiation of epithelial cells into mesenchymal cells has been widely described in other cell systems. In the kidney, the work of Strutz et al. suggested that PTC may express fibroblast specific markers in vitro and in vivo in a murine model of anti-TBM and anti-GBM model of nephritis (Strutz, 1995). More recent studies in 5/6 nephrectomised rats have demonstrated de novo expression of α-smooth muscle actin (α-SMA), a marker of myofibroblast phenotype, by PTC, associated with disruption of the tubular basement membrane (Ng et al., 1998). Furthermore more recent studies have identified a murine fibroblast specific marker, FSP1, which when transfected into murine PTC, led to a marked alteration in cell phenotype suggestive
of transdifferentiation (Strutz et al., 1995). Expression of this protein in murine proximal tubular epithelial cells can be induced by growth factors, such as TGF-ß1, and alterations in the extracellular matrix suggesting a role for these factors as potential modulators of renal epithelial transdifferentiation (Okada et al., 1997).

Recent studies have shown that stimulation of renal PTC with TGF-ß1 led to marked alteration in cell morphology, such that cells acquired an elongated, spindle shape, “fibroblastic” appearance associated with rearrangement of their actin cytoskeleton. In addition “activation” of the epithelial cell by the addition of TGF-ß1 was associated with induction of α-SMA mRNA and recruitment of actin into polymerisation of stress fibres. Despite these changes cells remained cytokeratin positive and desmin negative. Furthermore these changes were reversible on removal of TGF-ß1, suggesting that TGF-ß1 did not induce a stable alteration in cell phenotype. Although PTC expressed mRNA for type I collagen, alteration in cell morphology was not associated with changes in type I collagen mRNA expression. Similarly no stimulation of type I collagen protein synthesis could be demonstrated. In contrast alteration in PTC morphology in response to TGF-ß1 was associated with induction of type IV collagen mRNA and stimulation of collagen synthesis and its incorporation into the extracellular matrix. These studies therefore suggest that PTC are not the source of interstitial myofibroblasts, and also that TGF-ß1-activated PTC are not the source of the interstitial collagens which accumulate in the renal interstitium during progressive interstitial fibrosis.

In addition to its effect on PTC “activation” and matrix turnover PTC derived TGF-ß1 may also affect PTC cytokine generation. It has been demonstrated that TGF-ß1 stimulates FGF-2 generation by PTC is by stimulation of the secretion of pre-formed cytokine from within the cells (Jones et al., 1999a,b). FGF-2 in turn may also stimulate the secretion of pre-formed, latent TGF-ß1 by PTC suggesting a positive feedback loop involving TGF-ß1 and FGF-2, which may be involved in the progressive nature of renal fibrosis in vivo.

(b) Effect on fibroblast

Based on histological evidence that PTC injury precedes interstitial fibrosis (Knecht et al., 1991), and that regions of active interstitial fibrosis predominantly exhibit a peritubular rather than perivascular distribution (Alpers et al., 1994; Fine et al., 1995), it has been postulated that PTC relay fibrogenic signals to cortical fibroblasts in diseased kidneys. There is clear evidence to support this hypothesis, in that conditioned medium collected form non-stimulated PTC, may alter fibroblast mitogenesis and matrix synthesis (Lewis et al., 1996; Johnson et al., 1998; Lewis and Norman, 1998). In addition, TGF-ß1 regulates the terminal differentiation of fibroblasts and this is associated with induction of type I, type III and type IV collagen gene expression and protein deposition into the extracellular matrix. It is interesting to speculate therefore that PTC derived TGF-ß1 may be the key to the generation of the myofibroblast in the renal cortex in diabetes which subsequently mediates the progressive deposition of interstitial collagens.

Potential for cross-talk

Glucose also has the potential to stimulate the interstitial fibroblast to increase its synthesis of extracellular matrix and fibrillar collagens. This may be mediated by a direct effect on the cell (Jones et al., 1999a,b) or through an autocrine loop involving the induction of TGF-ß1 (Han et al., 1999). Furthermore the fibroblasts may also release factors which in turn stimulate PTC (Johnson et al., 1998). Thus TGF-ß1 and PDGF released from PTC enhanced the secretion of insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) from cortical fibroblasts. IGF-1 is believed to have an important role in modulating the progression of diabetic nephropathy (Sayeed-Ahmed et al., 1993; Wang et al., 1999, 2000; Rossert et al., 2000). It has also been shown to have a direct effect on PTC, including increasing phosphate transport into the cells (Silverstein et al., 2000). The conditioned medium from TGF-ß1-treated fibroblasts was subsequently shown to induce the proliferation and increased Na+/H+ exchange activity of PTC (Johnson et al., 1999). The active agent in the supernatants was identified as IGF-1 and the response could be reproduced by the addition of exogenous IGF-1 to the PTC. Furthermore the effect of IGF-1 was enhanced by IGFBP-3 implying a further method of control on this paracrine system. These observations not only implicate the PTC in the modulation of the function of adjacent fibroblasts but also demonstrate that the fibroblasts have the potential for a reciprocal effect on the function of the PTC. Furthermore we have recently reported that insulin affects the production of TGF-ß1 by PTC an effect mediated by signalling via the IGF-1 receptor (Morrisey et al., 1999a,b). This was a post-transcriptional effect involving a cytoplasmic protein binding to the 5' UTR of the TGF-ß1 mRNA transcript. There is therefore a great potential for reciprocal paracrine activation of PTC and fibroblasts.

The PTC is the predominant cell type in the normal renal interstitium, in which there are few resident interstitial fibroblasts. Adaptive changes in the renal cortex such as tubular cell hypertrophy and tubular membrane expansion, are a direct consequence of the alteration in the “glycaemic environment”. We hypothesise that the pathological changes in the diabetic renal interstitium associated with progressive renal insufficiency are initiated by glucose, which acts in combination with cytokines and pro-inflammatory stimuli. These cytokine-activated cells, however, are not a source of interstitial collagens. Furthermore the evidence supporting human PTC-myofibroblast
transdifferentiation is still incomplete. Activated PTC, however, do contribute to interstitial fibrosis indirectly by activation of interstitial fibroblast. Stimulated fibroblasts and myofibroblasts in turn may further amplify this response by their reciprocal action on the PTC. Further research in this area of PTC-interstitial fibroblast cross-talk may therefore hold the key to the understanding of the mechanisms that underlie the development of progressive renal fibrosis in diabetes mellitus.

References


Burg M., Piatik N., Green N. and Villey D. (1976). Organic solutes in amplify this response by their reciprocal action on the PTC. Further research in this area of PTC-interstitial fibroblast cross-talk may therefore hold the key to the understanding of the mechanisms that underlie the development of progressive renal fibrosis in diabetes mellitus.


Renal fibrosis and the proximal tubule in diabetes


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