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Invited Review

Diabetic nephropathy: the modulating influence of glucose on transforming factor ß production

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Summary. Diabetes in now the commonest cause of renal failure in the western world. Furthermore the survival of diabetic patients requiring dialysis treatment for renal failure is far less than patients with renal failure secondary to all other diseases. It is therefore important to identify the factors that control the development of progressive renal disease to allow targeted therapeutic interventions which would have major implications both to patient well-being and also to the provision of health care world wide. In this review we discuss possible metabolic consequences of hyperglycemia and their role in the pathogenesis of diabetic nephropathy. We also focus on the involvement of the pro-fibrotic cytokine Transforming Growth Factor ß, and contrast its role in the pathogenesis of glomerular and tubulo-interstitial changes seen in diabetic nephropathy.

Key words: Diabetes, TGF-ß1, Pathogenesis, Diabetic nephropathy

Diabetic nephropathy

Renal impairment is a common complication of both insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus. In 1985, it was estimated that 600 cases of end stage diabetic nephropathy occurred in the United Kingdom (10 cases per million population) (Joint Working Party on Diabetic Renal Failure of the British Diabetic Association, 1988), and that a third of all patients starting renal replacement therapy in the United States were diabetic (Eggers, 1988). Nearly 50% of patients with IDDM diagnosed before the age of 30 are affected, and of these two thirds will progress to end-stage renal failure.

Epidemiology

In IDDM the prevalence of nephropathy increases with duration of diabetes to a peak of 21% after 25 years. Following this, the prevalence declines to 10% in patients who have diabetes for 40 years (Andersen et al., 1983). This pattern of risk indicates that accumulation of exposure to diabetes is not sufficient to explain the development of clinically manifest kidney disease, and suggests that only a subset of patients are susceptible to renal complications. The cumulative incidence clearly indicates that only a proportion of IDDM patients will ever develop nephropathy. The ominous significance of renal involvement in IDDM is shown by the comparison of long term outcome in patients with and without nephropathy. Only 10% of patients with proteinuria survive after 40 years of diabetes, in contrast to more than 70% of those without proteinuria (Andersen et al., 1983).

Data on the prevalence and incidence of neprhropathy in NIDDM were sparse until recently. Unlike IDDM, the prevalence of proteinuria increases steadily with duration of NIDDM, from 10% in patients with less than 5 years diabetes to 20-35% in patients who have had diabetes for more than 25 years (Fabre et al., 1982; Klein et al., 1988a).

Clinically diabetic nephropathy initially presents with microalbuminuria, defined as albumin excretion ranging from 20-200 mg/24 hours. After a variable time period, the onset of the clinical phase of diabetic nephropathy is heralded by the appearance of persisted proteinuria (defined as a total protein excretion of 0.5g/day or more), which is followed by a progressive decline in glomerular filtration rate (GFR). The decline in GFR is linear over time leading to end stage renal failure. Because of this, diabetic nephropathy is the single most common cause of renal insufficiency in adults, accounting for 15-20% of patients starting renal replacement therapy each year (United States Renal Data System's 1990 Annual Data Report).

Metabolic pathogenesis of diabetic nephropathy

There is clear evidence of a positive relationship between hyperglycaemia and susceptibility to renal disease. Increased susceptibility to long term diabetic complications in patients with poor glycaemic control has been demonstrated in a number of epidemiological studies (Sosenko et al., 1984; Krolewski et al., 1985;

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Klein et al., 1988b; Chase et al., 1989; Kunzelman et al., 1989; The Diabetes Control and Complications Research Group, 1993). In addition, strict metabolic control in the very early stages of nephropathy may delay the development of microvascular complications of diabetes (Feldt-Rasmussen et al., 1986; Jorgensen et al., 1986; Reichard et al., 1993). Kidneys from non-diabetic donors develop typical changes of diabetic nephropathy when transplanted into diabetic recipients, thus supporting the role of the diabetic mileu in the development of renal injury (Mauer et al., 1983). However, as only 35-50% of all diabetics develop clinically apparent nephropathy, poor glycaemic control, although necessary, is unlikely in itself to be sufficient to initiate renal impairment.

It has been suggested that non-enzymatic glycation of proteins may have a role in the pathological changes observed in diabetes. Non-enzymatic glycation refers to a condensation reaction between glucose and the amino groups of lysine residues of protein amino acids. The reaction proceeds through formation of a Schiff base between the reducing group of glucose and amino acids. The resulting aldimine linkage is stabilised by undergoing an Amadori rearrangement to form a ketoamine. These glycosylation products when formed with proteins having long half lives undergo a slow complex series of chemical rearrangements to become irreversible advanced glycosylation end products (AGEs). The pathological consequences of these processes are unclear, although a number of possibilities have been suggested. Extracellular proteins such as collagen are rich in lysine, have a long half life and there is evidence that there is a three fold greater level of glycation of collagens from tissues of diabetic subjects. These reactions lead to increase collagen-collagen cross-linking which may interfere with matrix turnover (Brownlee et al., 1983). A number of cells including monocytes, fibroblasts and mesangial cells, have been shown to have AGE specific receptors. In addition during the removal of the AGE modified proteins, activation of these receptors on monocytes may lead to cytokine release (Vlassara et al., 1988), whereas activation on mesangial cells may lead to Platelet Derived Growth Factor (PDGF) release (Doi et al., 1992). Thus cytokine and growth factor release triggered by AGEs may have effects on cell proliferation and differentiation by autocrine or paracrine mechanisms, as well as modifying matrix turnover. However, at present the participation of non-enzymatic glycation, in the pathogenesis of diabetic nephropathy is un-proven.

There is a large body of experimental evidence that activation of the protein kinase C system and activation of the polyol pathway may both be mechanisms forming a link between hyperglycaemia and diabetic nephropathy (Bylander and Sens, 1990; Flath, 1992; Larkins and Dunlop, 1992; Bleyer et al., 1994; DeRubertis and Craven, 1994). Protein kinase C activation occurs as a result of the novo diacylglycerol synthesis generated as a result of increased glycolytic activity (Craven et al., 1990). Postulated harmful effects of polyol pathway activation involved in the pathogenesis of diabetes include accumulation of sorbitol, and alteration of the redox state of the cell due to the change in NAD/NADH ratio which accompanies conversion of sorbitol to fructose.

In addition to is metabolic effects, glucose modulates cell function via its increased osmolarity in solution. Exposure of cells to increase osmolarity results in an increase in the intracellular concentration of osmolytes which reduces cell shrinkage. This effect may be mediated by increased polyol pathway activation and polyol accumulation via induction of aldose reductase gene transcription, aldose reductase being the rate limiting enzyme for the polyol pathway (Bagnasco et al., 1987, 1988; Burg et al., 1996). Increased intracellular osmolytes may also occur as the result of induction of membrane transport proteins for other osmolytes such as betanine (Nakanishi et al., 1990; Uchida et al., 1993; Burg et al., 1996), or via the induction of facultative glucose transporters (Merrall et al., 1993; Itoh et al., 1994). Accumulation of osmolytes within the cell may itself result in modulation of cell function.

One possible explanation for the occurrence of nephropathy in less than half of all diabetic patients may be a genetic predisposition to develop nephropathy in a subgroup of patients. Supporting this concept is the occurrence of familial clustering of diabetic neprhropathy, and the increase incidence of diabetic neprhropathy in diabetic siblings of patients with diabetic nephropathy when compared to diabetic siblings of patients without nephropathy (Seaquist et al., 1989). The increased incidence of nephropathy among Pima Indians with type 2 diabetes mellitus is also cited as evidence for a genetic predisposition to nephropathy (Kunzelman et al., 1989). One possible explanation for an inherited predisposition to nephropathy comes from studies of sodium-lithium counter-transport activity in diabetic patients. Sodium-lithium transport activity is largely genetically determined and enhanced activity is associated with increased susceptibility to essential hypertension. Counter-transport activity is significantly elevated in diabetic patients with nephropathy who are thus genetically predisposed to develop hypertension as compared to diabetic patients without renal disease who have normal sodium/lithium counter-transport activity (Mangili et al., 1988). Poor glycaemic control is also related to the development of nephropathy in patients predisposed to develop hypertension as judged by a familial history of hypertension, and an increased sodium lithium counter-transport activity (Krolewski et al., 1988). It has, therefore, been proposed that the risk of development of renal disease in diabetes is associated with a genetic predisposition to hypertension and that this increases the susceptibility for renal disease in patients with poor glycaemic control (Krowleski et al., 1988).

Histopathology and structural - functional correlations

Most studies of the histological changes in diabetic

nephropathy have focused on the glomerular abnormalities culminating in diffuse glomerulosclerosis. The characteristic glomerular features being glomerular basement membrane thickening and diffuse increase in mesangial extracellular matrix, as originally described by Kimelsteil and Wilson (1936). Changes in the tubulointerstititum are not specific for a single disease process. Tubular cell hypertrophy occurs initially, followed by increased cellular proliferation. These compensatory changes occur early in the disease process and are later followed by tubular atrophy with interstitial fibrosis leading to a widening of the interstitium.

Studies by Mauer et al. on the structural-functional relationships in diabetic nephropathy demonstrated that mesangial expansion had a strong inverse correlation with glomerular filtration surface area and that this was correlated to the clinical manifestations of diabetic nephropathy (Mauer et al., 1984; Ellis et al., 1986). From this is was postulated that mesangial expansion leads to deterioration in glomerular function. Not all patients with mesangial expansion however, have nephropathy as defined as microalbuminuria. It is, therefore, uncertain whether glomerular mesangial expansion alone is the cause of renal insufficiency. In these studies the degree of cortical interstitial fibrosis was also noted to predict the clinical manifestations of diabetic nephropathy. More recently Mauer et al. have postulated that glomerular mesangial expansion and interstitial fibrosis are independent risk factors for the progression of renal disease (Lane et al., 1993).

The functional significance of histological changes in the renal cortical interstitium is now well established. Ridson et al. (1968) demonstrated, in a series of 50 patients with a variety of glomerulopathies, a highly significant correlation between the extent of tubular damage and creatinine clearance, and between plasma creatinine and the ability to concentrate urine. In contrast, there was a less significant correlation with the extent of the structural changes in the glomerulus. These initial findings have subsequently been confirmed by numerous studies of individual renal diseases (Schainuck et al., 1979; Mackensen-Haen et al., 1981, 1992; Wehrmann et al., 1989, 1990; Bogenschutz et al., 1990). This correlation between renal outcome and the degree of interstitial fibrosis is also true for diabetic nephropathy. Bohle et al. (1991) demonstrated a close correlation between the degree of interstitial fibrosis at the time of initial renal biopsy and renal outcome for more than 450 patients with diabetic nephropathy. In this study, severe cases of diabetic glomerulosclerosis were observed in the absence of tubulointerstitial changes. In such cases serum creatinine and creatinine clearance were normal. When mild mesangial sclerosis was accompanied by renal cortical interstitial fibrosis however, renal function was always abnormal (Bader et al., 1980). These observations have led to the hypothesis that changes in the interstitium may represent a crucial parameter which leads to progressive renal dysfunction.

Numerous hypotheses have been suggested to explain how tubulointerstitial fibrosis may contribute to the progression of renal dysfunction. As a result of widening of the interstitium, proximal tubules are separated from their blood supply and subsequently become atrophic (Mackensen-Haen et al., 1992). As a consequence there is an increased sodium load at the macula densa resulting in a reduction in renin secretion, efferent arteriolar vasodilatation and a reduction in glomerular filtration rate (Mackensen-Haen et al., 1981). Increase fibrosis also leads to tubular obliteration. As a result GFR is eliminated in the affected nephron and thus there is a decline in the total number of functioning nephrons.

With increasing awareness of the importance of pathological changes in the renal interstitium, increasing interest has focused on the role of non-glomerular cells, such as epithelial cells of the proximal tubule, in the initiation of a fibrotic response.

Structure and function of proximal convoluted tubule

In the renal cortex 85% of all cells are derived from the proximal convoluted tubule. This portion of the nephron extends form the glomerulus to the thin limb of the loop of Henle. Its average length is 15 mm and its diameter approximates 60 to 70 μm. Cells of the proximal tubule are continuous with the parietal epithelial cells of the glomerulus and the lumen is continuous with the urinary space. Proximal tubular epithelial cells are tall columnar cells which have a well developed brush border. This brush border results in a 40 fold increase in the surface area of the cells. Other characteristic features are the presence of tight junctions which form between cells, and large numbers of mitochondria, whose function it is to provide the energy source for fluid and electrolyte reabsorption.

In recent years it has become apparent that proximal tubular cells not only play an important role in sodium and water homeostasis, but have a potentially important role in inflammatory conditions of the renal cortex including interstitial nephritis and allograft rejection (Wuthrich et al., 1990; Fukatsu et al., 1993). To this end proximal tubular epithelial cells are a source of may cytokines including Transforming Growth Factor ß (TGF-ß), Endothelin, Tumour Necrosis Factor α (TNF-α), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), Monocyte Chemotactic Peptide-1 (MCP1), Interleukin 8 (IL-8), and Interleukin 6 (IL-6) (Kohan, 1991; Rocco et al., 1992; Schmouder et al., 1992, 1993; Yard et al., 1992; Frank et al., 1993; Boswell et al., 1994; Ong and Fine, 1994) as well as expressing receptors for a number of similar molecules (Norman et al., 1987; Humes et al., 1991; Quigley and Baum, 1991; Zhang et al., 1991; Yard et al., 1992; Cui et al., 1993). Proximal tubular epithelial cell function may also be modified by metabolic disturbance. It has been demonstrated that elevated glucose concentration leads to proximal tubular epithelial cell hypertrophy and increased collagen gene transcription *in vitro* (Ziyadeh et al., 1990). This, therefore, raises the possibility that

interactions between glucose and proximal tubular epithelial cells may contribute to the manifestations of diabetic nephropathy.

Possible mechanisms of tubulointerstitial fibrosis

The interstitium is comprised of a lattice of material, containing type I and III collagens in addition to fibronectin, non-collagenous glycoproteins and proteoglycans (Bohman, 1980; Ekblom et al., 1986). Accumulation of extracellular matrix may result from either increased production or decreased degradation of matrix components. Several factors, including cytokines and growth factors, have been implicated in the control of the fibrogenetic process and many of these may originated from cells native to the site of the inflammation. Renal tubular cells and glomerular cells produce a variety of polypeptide growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), and insulin like growth factors (IGF-I + II) which may affect the proliferation and matrix synthesis of a variety of cells (Hammerman, 1989; Kujubu and Fine, 1989; Guh et al., 1991; Fine et al., 1992; Floege et al., 1992; Frank et al., 1993; Verstepen et al., 1993). While it is likely that these factors have a role in the control of normal matrix turnover and tissue repair, dysregulated cytokine and growth factor synthesis may promote an alteration in the turnover of matrix proteins resulting in the fibrosis and scarring associated with progressive renal dysfunction. One of the more likely candidate growth factors participating in these events in Transforming Growth Factor ß (TGF-ß).

Mature TGF-ß1 which is functionally active, is a 25 kDa disulphide bonded homodimer (Rifkin et al., 1993). Natural TGF-ß1 is secreted in a biologically latent form, in which the mature molecule is associated with a disulfide linked dimer of the amino terminus of its precursor. The dimeric amino-terminal of the precursor is non-covalently associated with the mature TGF-ß1, and is also covalently associated through disulfide bonds with another protein of 125-160 kDa, which is a dimer of two 70 kDa polypeptides. This protein, which is structurally unrelated to the TGF-ß1 molecule, is called TGF-ß1 binding protein (LTBP). Antibodies to this binding protein inhibit in a dose dependent manner the activation of latent TGF-ß1 in co-culture experiments of endothelial and smooth muscle cells (Flaumenhaft et al., 1993). It has been postulated that this binding protein may be involved in the extracellular regulation of TGF-ß1 activation, possibly by concentrating the latent molecule on the cell surface where activation may then occur. There is evidence to suggest that the latent form of TGF-ß1 associates with the extracellular matrix via LTBP, and that the release of latent TGF-ß1 from the matrix is a consequence of proteolytic cleavage of LTBP (Lyons et al., 1988). In addition to its role in targeting and modulating TGF-ß1 activity, LTBP is also important in extracellular assembly of TGF-ß1, and in its efficient secretion (Miyazono et al., 1991). *In vitro* activation of latent TGF-ß1 can be achieved by transient acidification, alkalinisation or treatment with urea. Although the latent complex might be activated *in vivo* by exposure to acidic microenvironments, it seem more likely that it is activated by the action of proteases such as plamin and cathepsin D, both of which have been demonstrated to induce in vitro activation of the latent complex of TGF-ß1 (Lyons et al., 1988, 1990). The production of TGF-ß1 in a latent form is likely to be an important determinant for distribution of TGF-ß1 activity, ensuring that TGF-ß1 activity is only generated in the appropriate environment.

A major function of TGF-ß by interaction with its receptor, is to regulate the expression of genes whose products contribute to the formation and degradation of extracellular matrix (ECM). TGF-ß stimulates the production of collagen and fibronectin (Ignotz et al., 1987; Nakamura et al., 1992), thrombospondin (Penttinen et al., 1988), osteopontin (Noda et al., 1988), and proteoglycans (Bassols and Massague, 1988; Border et al., 1990a) and their incorporation into the extracellular matrix. It also increases the expression of integrins which are the cell surface receptors through which extracellular matrix proteins such as fibronectin and collagen interact with cells (Ignotz and Massague, 1987; Kagami et al., 1993). TGF-ß regulates genes encoding proteases and protease inhibitors, both of which effect turnover of the extracellular matrix. Generally TGF-ß leads to the accumulation of extracellular matrix by decreasing the synthesis of proteases and increasing the levels of protease inhibitors (Overall et al., 1989). In addition to controlling genes encoding for extracellular matrix components directly, TGF-ß is involved in the regulation of genes encoding for other growth factors. It can induce expression of both A and B chains of PDGF (Makela et al., 1987; Soma and Grotendorst, 1989; Battegay et al., 1990) and interleukin (IL)-1 (Wahl et al., 1987, 1993), and may inhibit expression of TNF-α and IFN-γ (Espevik et al., 1987). Therefore, by affecting the expression of these genes TGF-ß can indirectly alter many aspects of cell function. TGF-ß may also stimulate expression of its own gene in an autocrine manner, thus potentially amplifying its own signal (Van Obberghen-Schilling et al., 1988).

In renal disease TGF-ß has been implicated in directing fibrosis in both glomerular and interstitial compartments. In an experimental model of anti-glomerular basement disease in the rabbit, increased TGF-ß activity was associated with increased renal cortical collagen synthesis (Coimbra et al., 1991). A role for TGF-ß in directing interstitial fibrosis comes from the puromycin induced nephritis model in the rat in which increased expression of TGF-ß1 mRNA accompanies the histological manifestations of interstitial fibrosis, seen following repeated administration of purine aminonucleoside (Jones et al., 1991, 1992). More direct evidence for a pathogenic role for TGF-ß in which the administration of antibodies to TGF-ß at the time of induction of glomerular disease attenuates the histo-

logical manifestations of the disease (Border et al., 1990b).

Numerous studies have implicated TGF-ß as a possible mediator of the effects of elevated glucose concentrations on cells in tissue culture. Experiments using rat glomerular mesangial cells grown continuously in 30 mmol/l glucose for up to four weeks, showed increased production of fibronectin, laminin and type IV collagen (Ayo et al., 1990). In addition to increasing the production of extracellular matrix components, it was shown that increasing the glucose concentration in the culture medium increases the expression of TGF-ß mRNA (Wolf et al., 1992). More recently it has been demonstrated that the accumulation of extracellular matrix components in the glomerulus is mediated by autocrine production of TGF-ß (Ziyadeh et al., 1994). These studies have been cited as evidence for the involvement in TGF-ß in the pathogenesis of diabetic glomerulosclerosis.

An *in vivo* role for TGF-ß has recently been postulated in diabetic nephropathy (Yamamoto et al., 1993). Northern blotting and immunofluorescence for TGF-ß mRNA and TGF-ß protein respectively, performed in glomeruli of rats made diabetic by the administration of streptozotocin and in six patients with diabetic glomerulosclerosis. The results of this study point to an increase in both TGF-ß message and protein in the diabetic groups, which suggests a causative role for TGF-ß in diabetic glomerulosclerosis. Taken together these studies indicate that TGF-ß1 has a direct role in the glomerular changes associated with diabetic nephropathy.

In contrast to the studies on the pathogenesis of diabetic glomerulosclerosis, much less is known regarding the pathogenesis of the tubulointerstitial changes in diabetes. One of the earliest pathological changes in the tubulointerstitium in diabetes, is an acute increase in tubular basement membrane (TBM) mass which accompanies the development of renal hypertrophy (Ihm et al., 1992). We have demonstrated that exposure of cultured human proximal tubular epithelial cells to 25mM D-glucose increased type IV collagen and fibronectin in the culture supernatant (Phillips et al., 1997a). Furthermore we have demonstrated that accumulation of these basement membrane constituents involved an alteration in the degradative pathway, which was the result of a net increase in TIMP1 and TIMP2 activity over gelatinase activity. Specifically the addition of elevated concentrations of glucose caused an increase in TIMP 1, TIMP 2 and gelatinase A mRNA and protein expression.

Matrix production is controlled by a complex cytokine network. Of all cytokines studied to date TGF-ß1 has now emerged as a key mediator of matrix remodelling in health and disease. A role for TGF-ß1 cannot be inferred simply on the basis of alterations in mRNA, as it is now clear that TGF-ß1 synthesis may occur independently at the level of transcription, translation and secretion of pre-formed protein (Assoain et al., 1987; Villeger and Lotz, 1992; Phillips et al., 1995a, 1997b). Furthermore it is difficult to draw conclusions from studies in which TGF-ß1 protein is measured unless latent and active TGF-ß1 can be distinguished by the assay employed. Numerous studies have implicated TGF-ß1 in the extracellular matrix accumulation associated with diabetic nephropathy (Wolf et al., 1992; Ziyadeh et al., 1994). These in vitro studies however, utilised SV40 virally transformed cells, and it is known that transformation by SV40 may be accompanied by alterations in response to cytokines (Wang et al., 1996). Studies utilising non-transformed human endothelial cells demonstrated inhibition of cell proliferation and increased gene expression of basement components and TGF-ß1, on addition of high D-glucose concentrations (Cagliero et al., 1988a,b, 1991). Significantly there was no increase in active TGF-ß1, 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). In our studies glucose mediated accumulation of basement membrane constituents was dependent on polyol pathway activation and furthermore was not mediated by autocrine TGF-ß1 production (Phillips et al., 1997a).

Studies on the control of TGF-ß1 generation by proximal tubular cells have demonstrated that glucose caused an increase in TGF-ß1 mRNA, TGF-ß1 protein synthesis by proximal tubular cells was only seen following the subsequent application of a second stimulus (PDGF or ILß) (Phillips et al., 1995a, 1996). Addition of either of these cytokines increased the stability of D-glucose induced TGF-ß1 mRNA, as assessed by actinomycin-D chase experiments (Phillips et al., 1995b). In contrast incubation of D-glucose primed cells with TNF-α did not result in TGF-ß1 production, and interestingly unlike IL-1ß and PDGF, TNF-α, did not influence TGF-ß1 mRNA stability (Phillips et al., 1996). These observations suggest that post transcriptional modification of TGF-ß1 mRNA may be limited to specific cytokine stimulation, and may play a crucial role in the control of TGF-ß1 protein synthesis.

There is evidence to implicate both PDGF and TGF-ß1 in the pathological changes associated with diabetes mellitus. Serum from diabetic patients contains an enhanced growth promoting activity for vascular smooth muscle cells and for endothelial cells. It has been suggested that PDGF release from platelets is increased in diabetic patients, and that this may contribute to the growth promoting activity (Guillausseau et al., 1989). In addition, in vitro evidenced suggests that endothelial cells exposed to elevated glucose concentrations increase the expression of PDGF mRNA and secrete increased levels of PDGF protein (Mizutani et al., 1992). PDGF may therefore play an important role in the pathogenesis of diabetic angiopathy. In the mesangial cells of streptozotocin induced diabetic rats, increased mRNA for PDGF B-chains has been documented (Young et al.,

1995). The non-enzymatic glycation of proteins and the production of advanced glycosylation end products (AGEs) has been suggested to be a contributing factor in the pathological changes associated with diabetes. In the context of renal disease, mesangial cells have AGE specific receptors, and activation of these receptors leads to the release of PDGF (Vlassara et al., 1992). Evidence for the possible involvement of IL-1 comes from *in vivo* studies on streptozotocin induced diabetic rats, which demonstrated prominent glomerular macrophage infiltration, which is associated with increased expression of TGF-ß1 (Young et al., 1995). Furthermore studies of renal biopsies taken from patients with NIDDM have suggested that macrophages are involved in the initiation of the pathological changes of diabetic nephropathy (Furuta et al., 1993). In addition in other cell systems IL-1ß has been shown to induce the synthesis of TGF-ß1 (Offner et al., 1996). It can therefore be seen that both PDGF and IL-1 may be generated within the kidney in diabetes, and that their presentation to glucose primed proximal tubular cells may subsequently trigger the production of TGF-ß1.

It is interesting to speculate on possible mechanisms by which the addition of a second stimulus may alter the stability of D-glucose induced TGF-ß1 mRNA and hence facilitate its translation in HPTC. Possible explanations include, conformational changes in the tertiary structure of the mRNA, or alteration of the physical properties of the mRNA by protein binding induced by a second stimulus. Although no cytosolic binding proteins have been found that specifically regulate translation of TGF-ß1 mRNA, two untranslated regions of its mRNA have been postulated to be involved in its post transcriptional control, and hence might be the targets for IL-1ß or PDGF action. Analysis of the sequence of TGF-ß1 mRNA has identified an adenosine-uridine (AU)-rich region in its 3' unstralated region, which targets mRNA for rapid cytosolic degradation (Derynck et al., 1985). Cytosolic proteins have been identified which bind to this motif, and it has been shown that these proteins may be upregulated by stimuli, such as phorbol esters and/or calcium ionophore (Shaw and Kamen, 1986; Carter and Malter, 1991).

Recently another unstralated region at the 5' end of TGF-ß1 mRNA has been demonstrated to be involved in the post transcriptional control of TGF-ß1 synthesis (Kim et al., 1992). This sequence contains an unusually long untranslated region, with a high GC content. When inserted into the 5' unstralated region for human growth hormone it inhibits its production. Computer analysis has shown that this 5' untranslated region contains a stable secondary stem-loop structure. It is thought that duplex structures such as this, positioned close to the 5' end of mRNA inhibit initiation of translation by preventing binding of a 40S ribosomal subunit. Secondary structures present in the 5' untranslated region may serve as binding sites for cytoplasmic proteins which are able to modify its effect on the initiation of translation. Both of these untranslated regions of TGF-ß1 mRNA may therefore be potential sites for IL-1ß action leading to increased TGF-ß1 mRNA stability.

In vivo, proximal tubular cells (PTC) have an asymmetric distribution of transport proteins that facilitate the reabsorption of water, glucose and electrolytes from the glomerular filtrate. Sugar transport consists of two sequential processes. Movement from lumen to cell across the brush border is by Sodium-Glucose symporter activity (SGLT), and its exit from the cell occurs by facultative GLUT activity. Since these processes balance each other, glucose reabsorption by the kidney does not normally result in an increase in PTC intracellular glucose levels.

In culture, once confluent, proximal tubular cells form a polarised monolayer. By the use of tissue culture inserts, stimuli can be applied to either the apical or the basolateral aspect of the cells, and samples taken from either compartment for analysis. Utilising such a system we have demonstrated that the induction of TGF-ß1 mRNA by glucose, is a polarised phenomenon occurring only following the addition of glucose to the basolateral surface of these cells. Both glucose transport by GLUT1 and its metabolism are necessary, as the effect of glucose can be mimmicked by galactose but not by 2-deoxy D-glucose (Phillips et al., 1997c). A possible explanation for this may be that the induction of TGF-ß1 mRNA by glucose may occur as the result of increased intracellular glucose concentration due to a reversal of the GLUT 1 mediated glucose flux across the basolateral surface of the cell.

In summary, it can be seen that the pathogenesis of diabetic nephropathy is complex. It seems likely however that the interactions between glucose and the kidney are crucial for the initiation of pathological changes. Furthermore the response of cells within the glomerulus and the tubulointerstitium may differ in terms of the generation of the pro-fibrotic cytokine TGF-ß1 which is a key cytokine in the development of glomerulosclerosis and interstitial fibrosis within the kidney. It is also clear that although TGF-ß1 is undoubtedly a key factor in the pathogenesis of diabetic renal injury, glucose may have other direct effects on individual matrix components which also contributes to the histological appearance seen in diabetes mellitus.

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