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PERSPECTIVES IN RENAL MEDICINE

The tubulointerstitium in progressive diabetic kidney disease: More than an aftermath of glomerular injury?

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The tubulointerstitium in progressive diabetic kidney disease: More than an aftermath of glomerular injury?

Although the glomerulus, particularly the mesangium, has been the focus of intense investigation in diabetes, tubulointerstitial injury is also a major feature of diabetic nephropathy and an important predictor of renal dysfunction. The renal tubule in diabetes is subject to both direct and indirect pathogenetic influences as a consequence of its position in the nephron and its resorptive function. On exposure to glucose, proximal tubular cells elaborate vasoactive hormones, including angiotensin II and injurious cytokines such as transforming growth factor-β (TGF-β), as well as extracellular matrix proteins. In turn, angiotensin II may further increase TGF-β expression in both proximal tubular and interstitial cells, thus amplifying the stimulus to fibrogenesis in the renal tubulointerstitium. In addition to these mostly direct influences, the renal tubule, particularly its proximal segment, is exposed to glomerular effluent. In the diabetic state, this includes large quantities of advanced glycation end products and glucose and, at later stages in the evolution of diabetic nephropathy, protein, all of which are factors that may induce TGF-β expression and fibrosis. Diabetic nephropathy should therefore be viewed as a disease affecting the entire nephron. Continued exploration into tubulointerstitial disease in addition to glomerular injury in diabetes may help provide further insights into the pathogenesis of diabetic nephropathy and additional targets for therapeutic intervention.

The tubulointerstitium of the diabetic kidney has become a major focus of study, with considerable advances having been made in the understanding of its role in the pathogenesis of diabetic nephropathy over the last decade [1, 2]. In particular, recent *in vivo* studies have confirmed the observations originally made in cell culture, highlighted the complexities of cell interactions within the tubulointerstitial compartment, and shown it to be an important site of action for renoprotective therapies in diabetic kidney disease.

Key words: tubule, interstitium, transforming growth factor β, extracellular matrix.

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ARCHITECTURE OF THE INTERSTITIUM

The tubulointerstitium encompasses the tubular epithelium, vascular structures, and interstitium, together accounting for more than 90% of the kidney volume [3]. The availability of antibodies that are relatively specific for different cell types in combination with electron microscopy has led to a more precise identification of resident cell populations in the interstitium of the healthy kidney [4, 5]. Fibroblasts are distinguished from other interstitial cells types by the presence of ecto-5′-nucleotidase, junctional complexes, and prominent subplasmalemmal actin filaments. Fibroblasts constitute the major cell type in the interstitium, where they interconnect with tubules, vessels, and each other to provide a scaffold-like structure. The remaining interstitial cells—dendritic cells, lymphocytes, and macrophages—are related to the immune system. Antigen-presenting dendritic cells are present in the peritubular space and are particularly abundant in the inner stripe of the outer medulla. Macrophages are mostly confined to the adventitia of large blood vessels, whereas lymphocytes are rarely present in normal kidney. Although incompletely defined, particularly in the diabetic context, the kidney's fibrogenic response to other types of nonimmune-related injury such as renal mass reduction involve major changes in two interstitial cell populations: fibroblasts and macrophages. The appearance of active fibroblasts, which express α-smooth muscle actin (myofibroblasts), is associated with the production of fibrillar collagens [6]. These activated interstitial fibroblasts may then interact with tubular epithelial cells in bidirectional "cross-talk" [7] and may also undergo a process referred to as "transdifferentiation" to also become fibroblastic-like [8]. However, it remains unclear whether or not these changes indicate a complete change in cell phenotype or rather an alteration in the level of expression of only certain proteins. The response of the kidney to non-immune–mediated injury also includes an increase in macrophages providing a rich source of various cytokines, including the profibrotic transforming growth factor-β (TGF-β) [9]. Although a contribution from circulating cells cannot

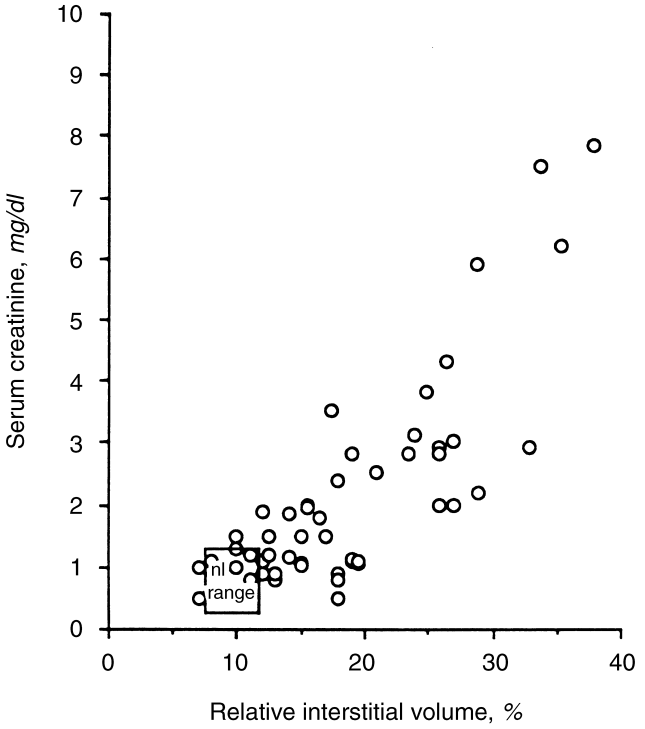

Fig. 1. Relationship between serum creatinine concentration (vertical axis) at the time of biopsy and relative interstitial volume (horizontal axis) in diabetic nephropathy. The normal (nl) range is indicated by the square. (Reprinted with permission from the International Society of Nephrology [1] and *Pathology Research and Practice* 167:204–216, 1980 [16].)

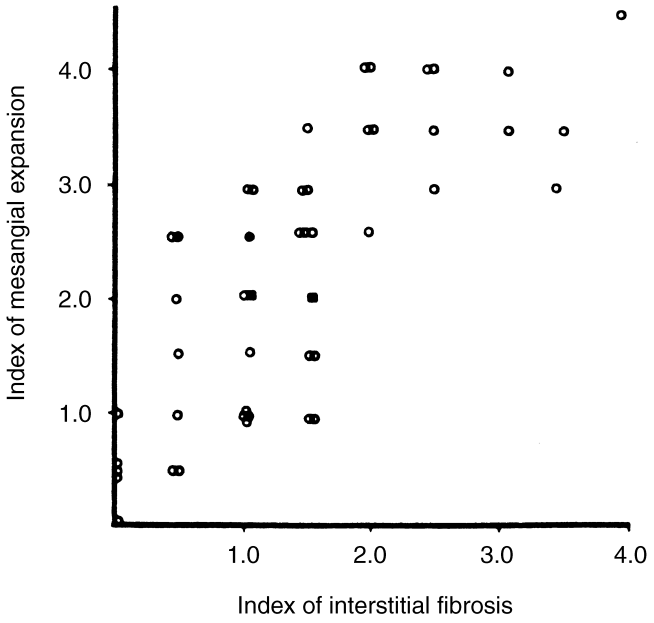

Fig. 2. Correlation between indices of mesangial expansion and interstitial fibrosis in patients with type I diabetes ($r = 0.80$, $P < 0.0005$). (Reprinted with permission from the *Journal of Clinical Investigation* 74:1143–1155, 1984 [18].)

be excluded, recent studies indicate that local proliferation of both macrophages and myofibroblasts is a prominent feature of progressive renal injury [10].

Recent insights into the mechanisms of progressive renal dysfunction have indicated that tubulointerstitial pathology is not simply an aftermath of glomerular injury, but that tubular cells may be primary targets for various pathophysiological influences [11, 12]. Indeed, in diabetes, perturbations in glucose-dependent metabolic pathways and vasoactive hormone systems may directly influence both tubular and interstitial cell behavior and ultimately lead to interstitial fibrosis and renal dysfunction caused by nonglomerular mechanisms [13, 14].

PATHOLOGICAL CHANGES

The extent of tubulointerstitial injury correlates closely with long-term renal function in a variety of primary glomerular diseases [15]. Pathological changes that have been described in association with diabetic nephropathy include thickening of the tubular basement membrane (TBM), tubular atrophy, interstitial fibrosis, and arteriosclerosis. In particular, interstitial expansion correlates closely with the magnitude of renal dysfunction, albuminuria [16, 17] (Fig. 1), and mesangial enlargement in both type I (insulin-dependent) (Fig. 2) [18] and type II (non-insulin–dependent) diabetes [19]. Moreover, although a close correlation between various structural parameters was noted in a study of 84 patients with type I diabetes, stepwise multiple regression analysis found that the impact of interstitial volume was additive to that of mesangial expansion in its relationship to renal function, suggesting an independent effect [17]. Furthermore, in a four-year longitudinal study in patients with overt nephropathy in which structural injury was assessed histomorphometrically at both study entry and completion, interstitial fibrosis rather than glomerular injury correlated most closely with declining creatinine clearance (Fig. 3) [20].

In contrast to these interstitial changes, the relationship between TBM width and kidney function is uncertain, although its correlation with glomerular basement membrane (GBM) thickening (Fig. 4), itself not a good predictor of renal impairment [18], suggests that changes in TBM may reflect glycemic exposure rather than evolving nephropathy [21].

LINK BETWEEN GLOMERULAR AND TUBULOINTERSTITIAL INJURY

The mechanisms whereby tubulointerstitial fibrosis develops in association with glomerular injury have been the subject of vigorous speculation following the appreciation of the role of the tubulointerstitium in disease progression. Kriz et al have categorized the proposed mechanisms

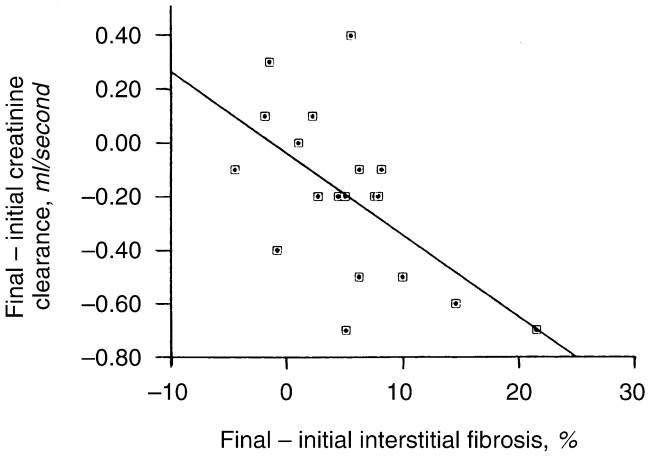

Fig. 3. Association between the fall in creatinine clearance and rise in renal cortical interstitial fibrosis in hypertensive diabetic subjects who underwent renal biopsies at the commencement and completion of the four-year study ($r = -0.591$, $P = 0.008$). (Reprinted with permission from *Diabetes* 43:1046–1051, 1994 [20].)

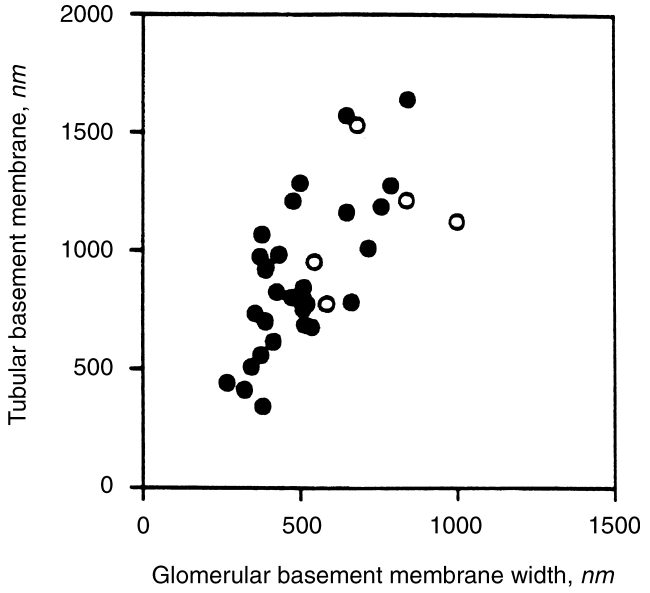

Fig. 4. Relationship between tubular basement membrane (TBM) width and glomerular basement membrane (GBM) width in normotensive (●) and hypertensive (○) patients with type I diabetes ($r = 0.67$, $P < 0.001$). (Reprinted with permission from the International Society of Nephrology [21].)

into three nonmutually exclusive groups: (*a*) self-sustaining prosclerotic cytokine production in both the glomerulus and tubulointerstitium; (*b*) excessive protein load to the proximal tubule, ultimately leading to peritubular inflammation and fibrosis; and (*c*) postglomerular vasoconstriction with peritubular capillary rarefaction, tubular ischemia, and atrophy [22]. As detailed in this review, evidence of all three mechanisms has been documented in diabetic nephropathy. More recently, on the basis of sequential structural studies a fourth mechanism has also been proposed as a pathogenetic link between glomerular and tubulointerstitial injury: the interstitial spread of glomerular filtrate [22]. In a rat model of focal segmental glomerulosclerosis, glomerular injury was associated with misdirection of filtrate in to the periglomerular and peritubular space leading to the development of periglomerular and peritubular fibrosis. A final degeneration of the nephron with the loss of filtration may then occur not only as a consequence of concurrent glomerular and tubulointerstitial injury, but also when tubular degeneration progresses more rapidly than glomerular fibrosis, leading to the development of atubular glomeruli with open capillary loops. Thus, in progressive renal disease, GFR may fall as a result of glomerular fibrosis or as a consequence of disconnection of the glomerulus from normal proximal tubules [23]. In diabetic nephropathy, the presence of such atubular glomeruli has been well documented [22, 24], although their proportion in relationship to globally sclerotic glomeruli has not yet been determined. However, in the remnant kidney, a model of glomerular hemodynamically-mediated renal injury with functional similarities to diabetes [25], atubular rather than globally sclerotic glomeruli predominate [23].

The finding that both tubulointerstitial and glomerular lesions develop in diabetes is not unexpected given the exposure of both regions to similar factors in the diabetic milieu. However, there are some important differences in the extent to which both sites are exposed to various biochemical and hemodynamic factors. These differences may explain the findings of Bader et al, who in a study of 103 patients with varying degrees of glomerulosclerosis reported that in addition to a significant relationship between cortical interstitial volume and serum creatinine, there was a dissociation between glomerular injury and renal dysfunction in a number of patients [16]. In these cases, severe glomerular lesions were accompanied by a normal serum creatinine if the interstitium showed no fibrotic change, and conversely, mild glomerular lesions accompanied by interstitial fibrosis always had elevated serum creatinine concentrations. More recently, in a study of microalbuminuric type II diabetic patients, Fioretto et al reported that a pattern of predominant tubulointerstitial disease was the most common histological type, being present in 41.2% of patients studied, whereas parallel glomerular and tubulointerstitial changes were found in 29% [26]. However, how such differences in histopathology relate to the finding that a subgroup of diabetic patients may show a decline in renal function without increasing albuminuria remains uncertain [27, 28].

Like the human, tubulointerstitial pathology is also a feature of experimental diabetes where changes include tubular dilation and expansion of the interstitial space [1, 29]. Similar to the findings in the diabetic glomerulus

[30], the prosclerotic cytokine TGF-β has been implicated in the development of tubulointerstitial pathology, as indicated by increased expression of its mRNA, protein, and biological activity at this site [29, 31, 32].

RENAL ENLARGEMENT

An increased kidney size was first described in diabetes more than a century ago [33]. The nature of this nephromegaly has been characterized in detail in elegant studies using stereological techniques [34, 35]. Because glomeruli account for only a small fraction (less than 10%) of kidney mass, renal enlargement in diabetes predominantly reflects tubulointerstitial changes. In the streptozotocin diabetic rat, the first seven weeks following the induction of experimental diabetes are accompanied by a 37% increase in proximal tubular length, a doubling of wall volume and luminal diameter, and an increase in cell height [36], reflecting both hypertrophy and hyperplasia [37]. This renal enlargement is associated with an increase in glomerular filtration rate (GFR). However, the finding that good glycemic control ameliorates glomerular hyperfiltration but not nephromegaly in patients with type I diabetes suggests a dissociation between glomerular and tubular components [38]. Other investigators have reported partial reduction in kidney size in diabetic patients after improved glycemic control with intensified insulin therapy [39]. Like hyperfiltration [40], the significance of nephromegaly as a predictor of subsequent nephropathy is uncertain, although recent data suggest that renal enlargement may portend a poor prognosis in patients with type I [41] as well as type II [42] diabetes.

In animal studies examining extracellular matrix expression in the four weeks following the induction of experimental diabetes, basement membrane type collagen α1 (IV) gene expression was noted in the proximal tubule [32, 43]. Changes in glomerular collagen expression were also noted at this time in some, but not all studies, in early experimental diabetes [32, 43, 44]. In addition to quantitative changes in type IV collagen expression in the diabetic kidney, qualitative changes have also been observed in both experimental and human diabetes with the appearance of nonclassic α chains in addition to the usual chains 1 and 2 [45, 46].

A number of cytokine systems in which both ligand and receptor are present in tubules have been implicated in the pathogenesis of renal enlargement in diabetes [47]. These include insulin-like growth factor-I (IGF-I) [48], epidermal growth factor (EGF) [49], and TGF-β [32]. In the BB rat and NOD mouse, increased TGF-β immunostaining was noted as early as three days following the onset of diabetes, where it was localized predominantly to renal tubular cells [50]. Furthermore, neutralizing anti–TGF-β antibodies ameliorated renal hypertrophy and the overexpression of matrix protein mRNA in the kidney cortex [51], the bulk of which consists of proximal tubules. These findings suggest that TGF-β may be an important growth factor in tubular as well as glomerular growth in diabetes, in addition to its role in the later development of glomerulosclerosis and tubulointerstitial fibrosis [52].

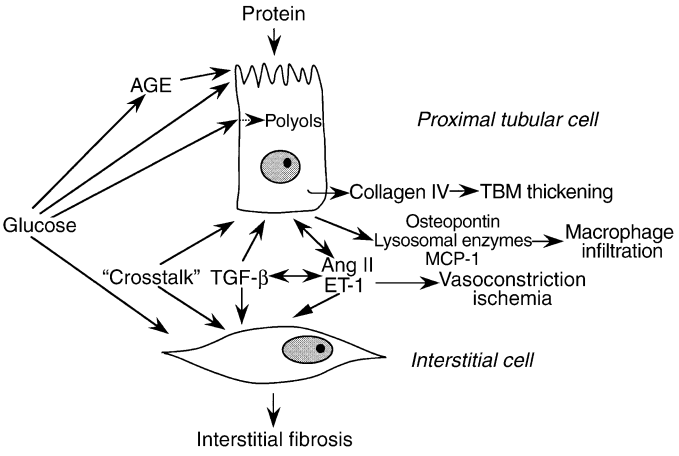

Fig. 5. A schema suggesting a complex series of interactions between interstitial and proximal tubular cells involving vasoactive hormones, glucose-dependent pathways such as polyols and advanced glycation end products (AGEs), and cytokines such as transforming growth factor-β (TGF-β), leading to tubular basement membrane thickening, macrophage infiltration, and tubulointerstitial fibrosis.

Pathogenesis of tubulointerstitial changes

The finding that cultured tubular [53] and interstitial cells [54] respond to glucose suggests that these cells contribute directly to the pathological changes of diabetic nephropathy rather than as a consequence of glomerular injury. Moreover, the recognition of bidirectional communication between these two cell types [7] along with the potential fibroblastic transformation of tubular epithelial cells [8] and the infiltration of mononuclear cells [55] together highlight the complexity of local fibrogenic pathways in the tubulointerstitium. Thus, although the tubular epithelial cell synthesizes TBM and the interstitial fibroblast may be largely responsible for interstitial fibrosis, the interaction between the cellular components of the tubulointerstitium is likely to be an important determinant of the pathology observed. Furthermore, the pathophysiological changes that develop in the setting of diabetes are not confined to hyperglycemia, but include alterations in vasoactive hormones, formation of advanced glycation end products (AGEs), hemodynamic changes, and activation of various secondary metabolic pathways [14] leading to oxidative stress, protein kinase C activation, and increased polyol production (Fig. 5) [56].

GLUCOSE

Proximal tubular cells, similar to cells at other sites of diabetic complications, do not have an absolute requirement for insulin for glucose uptake [57], so that the intracellular glucose level is directly related to its plasma concentration. Elegant studies by Ziyadeh et al have demonstrated increased steady-state levels of mRNA for collagen types IV and I in cultured proximal tubular cells exposed to high glucose concentrations, possibly reflecting activation of an enhancer sequence within the procollagen gene [53]. In addition to increased glucose in plasma and interstitial fluid, excess glucose in the glomerular filtrate in diabetes leads to enhanced proximal tubule glucose resorption, further augmenting the effects of hyperglycemia on intracellular glucose flux within the proximal tubule [58]. However, despite the increased activity of the brush border glucose transporter, GLUT-5 in diabetes [59], the synthesis and secretion of TGF-β is mostly dependent on basolateral glucose exposure [60], suggesting that it is the interstitial rather than the urinary glucose concentration that modulates the expression of this profibrotic cytokine.

POLYOL PATHWAY

A role for the hyperglycemia-induced acceleration of polyol pathway metabolism in mediating the development of diabetic nephropathy has been suggested by some investigators [61]. The increased formation and accumulation of sorbitol occurs via metabolism of glucose by the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme aldose reductase. Sorbitol accumulation may, in turn, lead to depletion of free myoinositol, loss of Na^+,K^+-ATPase activity, and increased consumption of the enzyme cofactors NADPH and NAD^+, leading to changes in cellular redox potential and cellular dysfunction [61]. In cultured proximal tubular cells, incubation in a high-glucose medium results in increased sorbitol [56]. Glucose-induced stimulation of extracellular matrix expression in proximal tubular cells *in vitro* could be abrogated by both myoinositol supplementation and aldose reductase inhibition [56, 62]. However, despite the beneficial effects of aldose reductase inhibition in both proximal tubular [56] and mesangial cells [63] in culture, long-term experimental studies have been conflicting with respect to effects on structural and functional indices of injury in diabetic nephropathy [64, 65], possibly reflecting the incomplete blockade of aldose reductase activity with the drugs used. The effects of aldose reductase inhibition on tubulointerstitial injury in experimental diabetes have not been specifically examined.

ADVANCED GLYCATION END PRODUCTS

Evidence has accumulated over the last decade implicating the formation of AGEs as a major factor in the pathogenesis of diabetic nephropathy [66]. Recently, it has been demonstrated that AGEs can activate a range of intracellular second messengers, including mitogen-activated protein kinase in a renal tubular cell line [67], thereby leading to the induction of expression of various cytokines including TGF-β [68]. Glycated proteins are preferentially excreted in the urine from both control and diabetic rodents [69], although the relative roles of glomerular filtration and proximal tubular resorption on this phenomenon have not been elucidated. Studies in experimental diabetes have shown AGE accumulation in renal tubules and its amelioration by treatment with the inhibitor of advanced glycation, aminoguanidine [70]. As with other long-lived protein structures, the TBM undergoes advanced glycation in diabetes [71], but in addition, the proximal tubule is also a site of catabolism of the increased circulating AGEs found in diabetes [72, 73], presumably reflecting their filtration and absorption at this site [72]. Indeed, it has recently been demonstrated that AGE binding to proximal tubules is increased in diabetes [74]. Furthermore, concomitant assessment of renal AGE levels suggested that their local concentration regulated their own binding to proximal tubules with aminoguanidine, attenuating the increased AGE binding in diabetes (Fig. 6) [74].

Whether or not Amadori products, which are intermediates in the formation of AGEs that have also been implicated in the pathogenesis of diabetic nephropathy [75], are taken up by the proximal tubular epithelium or influence tubulointerstitial injury has not been delineated.

VASOACTIVE HORMONES

Despite suppression of the systemic renin-angiotensin system in diabetes, the ability of angiotensin-converting enzyme (ACE) inhibition and angiotensin II type 1 receptor (AT_1) antagonism [76] to ameliorate renal injury in both experimental and human diabetes has implicated the local intrarenal renin-angiotensin system in the pathogenesis of diabetic nephropathy [77]. Quantitation of the various components of the renin-angiotensin system (RAS) in kidneys of experimental animals has yielded conflicting results, possibly reflecting the low levels of expression outside the juxtaglomerular apparatus [77]. However, with the use of ultrasensitive techniques such as reverse transcription-polymerase chain reaction (RT-PCR), low levels of renin mRNA have been detected in the microdissected tubules of normal rats [78], and in addition, modulation of renin expression in response to uninephrectomy [78], salt depletion [79], and ACE inhibition [80] has also been noted. Furthermore, in a recent study of patients with diabetic nephropathy, increased expression of renin, ACE, and angiotensinogen were noted in the proximal tubule [81]. *In vitro* studies have shown that angiotensin II may act synergistically

with glucose [82] and EGF [83] to induce hypertrophy. More recently, *in vivo* studies in the renin-overexpressing Ren2 rat have shown that an induction of diabetes leads to severe tubulointerstitial fibrosis, as well as glomerulosclerosis, pathological changes that are both ameliorated by ACE inhibitor therapy [84].

Angiotensin II, in addition to its hemodynamic effects, may also act as a growth factor [85], potently inducing the expression of TGF-β in a variety of cell types, including both proximal tubule epithelial cells [86] and renal interstitial fibroblasts [87]. Both glucose and angiotensin II promote TGF-β1 expression in a range of cells types, including those of the proximal tubule. Recent studies suggest an interaction between these two stimuli, with *in vitro* studies indicating that glucose promotes expression of the precursor of angiotensin II, angiotensinogen [88], as well as the AT_1 [89]. This provides an additional mechanism whereby the effects of chronic hyperglycemia on proximal tubular cytokine production and extracellular matrix accumulation are amplified. In long-term diabetic rats, ACE inhibition was associated with a reduction in tubular injury and a diminution in TGF-β1 and type IV collagen overexpression, particularly in the proximal tubule (Fig. 7) [29]. Furthermore, in a study performed in uninephrectomized, alloxan-induced diabetic (DM) beagle dogs, a protective effect on tubulointerstitial injury was similarly observed with the ACE inhibitor lisinopril [90]. More recently, in a study of patients with type 2 diabetes and nephropathy, sequential biopsies revealed a 30% increase in cortical interstitial volume over a two-year period [91]. In contrast, no change in cortical interstitial volume was noted in patients treated with the ACE inhibitor perindopril. Although mesangial volume also changed in the same direction as the cortical interstitium, it did not reach statistical significance.

Another vasoconstrictor and trophic hormone, endothelin, also has potent actions within the kidney. Endothelin expression has been reported to be up-regulated in glomeruli [92] and whole kidney extracts [93] in diabetic rats. More recently, endothelin receptor antagonism was reported to not only influence glomerular injury in experimental diabetes, but also to attenuate endothelin overexpression in renal tubules [94].

SALT AND HYPERTENSION

In addition to the well-described association between overt nephropathy and hypertension, type 1 diabetic patients with incipient nephropathy have higher blood pressure over a 24-hour period compared with normoalbuminuric patients [95]. The development of elevated blood pressure in association with such early kidney disease signifies the intimate relationship between blood pressure and the evolution of diabetic nephropathy.

Hydrostatic forces associated with elevated blood pressure may be transmitted to the peritubular capillary network that, being devoid of pericytes and smooth muscle, is less well adapted than the glomerulus to deal with the increased pressure [96]. In experimental studies in which systemic blood pressure is increased by angiotensin II infusion, interstitial myofibroblasts, a source of various cytokines including TGF-β [9], encircle peritubular capillaries [97]. It has been suggested that the accrual of these smooth muscle-like cells might lead to a dampening in pressure transmission along the peritubular capillary network, resulting in a reduction in pressure natriuresis and salt-dependent hypertension [97]. These tubulointerstitial changes may in part explain the increase in exchangeable sodium and extracellular volume that develops in association with diabetes [98]. Indeed, salt, *per se*, in the absence of hypertension, may lead to fibrotic change by TGF-β–dependent mechanisms [99].

PROTEIN TRAFFIC

As a consequence of the abnormal glomerular permeability that develops in diabetic nephropathy, increased quantities of filtered plasma proteins reach the proximal tubule. These proteins include albumin and potentially more toxic proteins such as IgG, transferrin, lipoproteins, and complement components that are then endocytosed by the tubular epithelium and degraded in lysosomes. With heavy proteinuria, lysosomal capacity is exceeded, leading to lysosomal rupture and phenotypic changes in the tubular epithelium that include the expression and basolateral release of the potent vasoconstrictor endothelin and the mononuclear cell chemotactic proteins: macrophage chemotactic protein-1 (MCP-1) and osteopontin [100]. These vasoactive and chemotactic factors, in turn, may lead to ischemia, overexpression of proinflammatory and fibrotic cytokines, and infiltration of mononuclear cells [101]. The contribution of protein overload to interstitial pathology in diabetes may explain why fibrosis in this region is a prominent feature of progressive disease in late [20] but not early disease [102]. In addition, the tubular toxicity of protein raises the possibility that the beneficial effects of ACE inhibitors in diabetic renal disease [103] may reflect their potent antiproteinuric action [104] in addition to the reduction of angiotensin II-mediated effects on growth factor activation and glomerular hemodynamics.

ISCHEMIA

The role of ischemia in the pathogenesis of tubulointerstitial injury in diabetic nephropathy has not been examined in detail, despite the recognition of arteriolar pathology as a characteristic feature of the disease and its association with both glomerular and tubulointerstitial injury [16, 26]. Tubulointerstitial ischemia in diabetes may

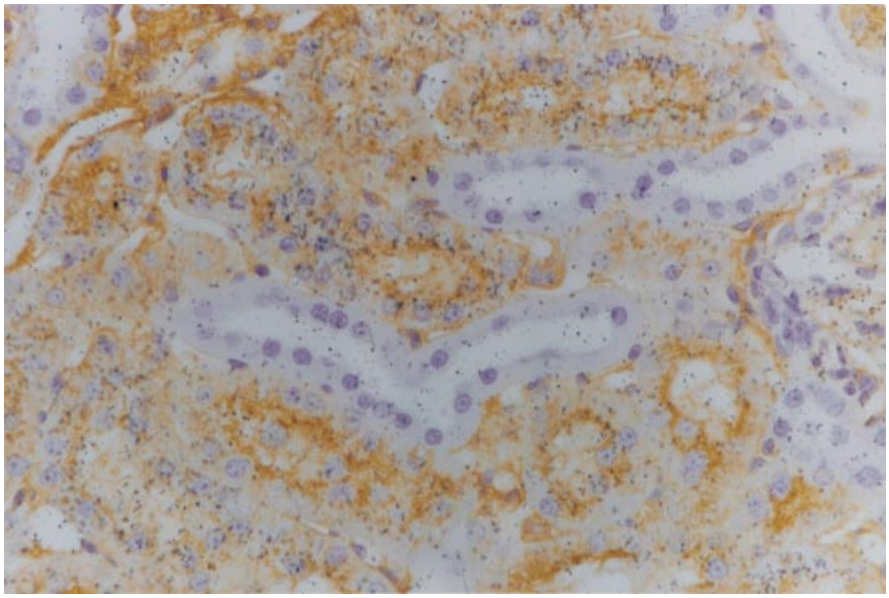

Fig. 6. Bright field *in vivo* emulsion autoradiograph with lectin counterstain showing ^{125}I-AGE-BSA binding to proximal tubules but not glomeruli or distal tubules. (Reprinted with permission from the International Society of Nephrology [74]; magnification ×400).

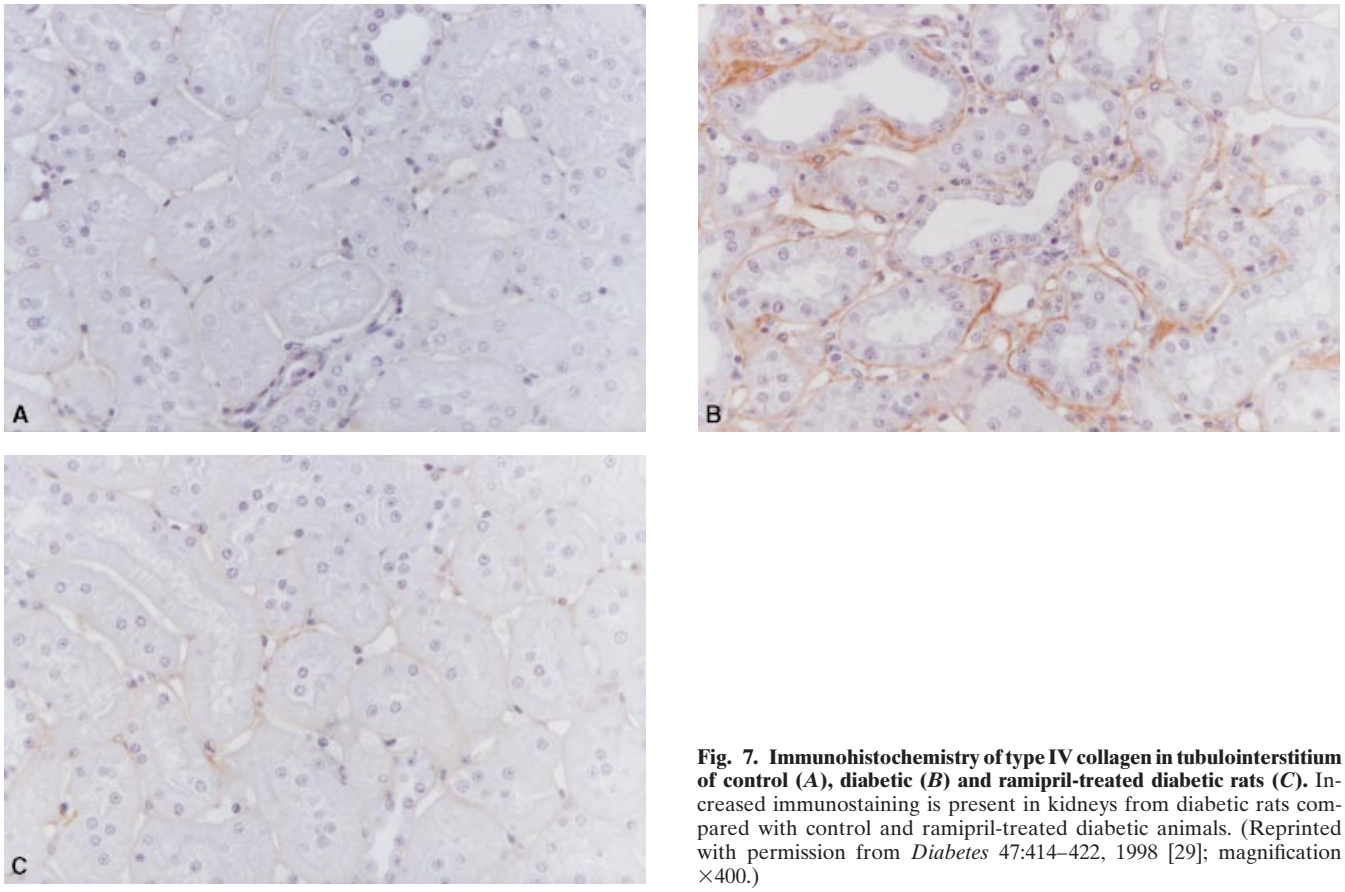

Fig. 7. Immunohistochemistry of type IV collagen in tubulointerstitium of control (*A*), diabetic (*B*) and ramipril-treated diabetic rats (*C*). Increased immunostaining is present in kidneys from diabetic rats compared with control and ramipril-treated diabetic animals. (Reprinted with permission from *Diabetes* 47:414–422, 1998 [29]; magnification ×400.)

develop as a result of increased metabolic demands of surviving tubules [105] or as a consequence of reduced peritubular flow in the setting of postglomerular vasoconstriction, glomerular nonperfusion, or capillary obliteration following tubulointerstitial expansion. The *pars recta* or S3 segment of the proximal tubule may be particularly vulnerable to nephrotoxic and ischemic injury as a consequence of tubular concentration, interstitial hypertonicity,

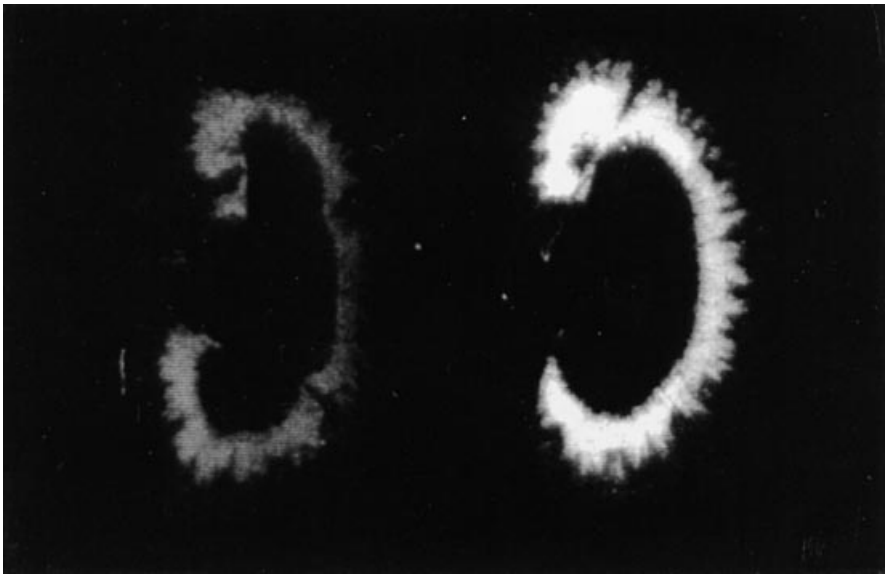

Fig. 8. *In situ* hybridization for βig-h3 mRNA, a marker of TGF-β biological activity, in longitudinal sections of control (left panel) and diabetic (right panel) rat kidney localizing gene expression to the inner cortex and outer stripe of outer medulla. (Reprinted with permission from the International Society of Nephrology [31].)

and low oxygen tension in this region [106]. This has led to the suggestion that ischemic peritubular microangiopathy in diabetes may preferentially affect this region of the nephron [107]. Indeed, the *pars recta* was the predominant site of collagen overexpression [43] and a major site of TGF-β biological activity in the diabetic rat (Fig. 8) [31]. Furthermore, in a study of patients with incipient diabetic nephropathy, Nuyts et al reported a significant correlation between glycated hemoglobin, a marker of the rate of progression of early nephropathy [108], and urinary excretion of the intestinal alkaline phosphatase (hIAP), the isoenzyme expressed exclusively by the *pars recta* of the proximal tubule [107].

SUMMARY

Tubulointerstitial injury is a major feature of diabetic nephropathy and an important predictor of renal dysfunction. Its development may reflect influences that are common to other forms of renal disease but also those that are unique to diabetes. Primarily glomerular factors, such as protein leakage, are likely to be important contributors to progressive tubulointerstitial injury in diabetes and to explain in part the beneficial effects of ACE inhibitors as antiproteinuric and renoprotective agents in both diabetic [103] and nondiabetic kidney disease [109]. The diabetic state with its associated increased production of glucose, polyols, and AGEs may lead not only to glomerular injury, but also to tubulointerstitial damage by the activation of various, ultimately injurious pathways following their mostly unimpeded transglomerular passage. The activation of local vasoactive hormone systems, especially the RAS in the tubulointerstitial compartment, may directly lead to tubulointerstitial fibrosis and damage to the peritubular capillary network with the tubule and in particular the *pars recta* of the proximal tubule being especially vulnerable. Thus, rather than a solely glomerular disease, diabetic nephropathy develops as a consequence of complex interactions between the glomerulus, tubule, interstitium, and vascular components of the kidney. Continued exploration into the pathogenesis of nonglomerular as well as glomerular disease may help provide additional targets for therapeutic intervention in diabetic nephropathy.

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