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From Bedside to Bench and Back Again: What Animal Models Teach Us
About Renal Disease and What They Don't

Paradigms in Diabetic Nephropathy (DN)
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1. Animal models of DN can be useful, even if imperfect, if they generate testable concepts in humans.
2. In order to be useful, these models:
 - a. Need to reflect components of the structural, functional and natural history, human conditions which are well understood.
 - b. Should recapitulate the order of the human disorder so that the highly disease specific processes involved in the genesis of the early lesions are not confused with the progression promoters associated with advanced injury, which share commonalities among multiple renal disorders.
3. An example of a concept that has been distorted, in the end, by the interpretations provided to animal models of DN (i.e., hyperfiltration/capillary hypertension/renin angiotensin system blockade) resulting in inappropriate extrapolation to humans will be provided.
4. A review of human diabetic nephropathology, natural history, and structural functional relationships and examples of useful animal models will be presented.

The Histology of Progressive Diabetic Nephropathy in Humans

The constellation of the renal structural lesions occurring in diabetes is unique, although many of these lesions can be individually observed in other renal disorders. The morphologic lesions in type 1 diabetes (T1DM), predominantly affect the glomeruli, with thickening of glomerular basement membrane (GBM) and mesangial expansion, although also the podocytes, renal tubules, interstitium and arterioles undergo substantial changes, especially at later stages of disease (1-5).

GBM thickening, the first measurable change, has been detected as early as 1.5 to 2.5 years after the onset of T1DM (6, 7). Thickening of tubular basement membrane (TBM) closely parallels that of GBM thickening (3). Mesangial expansion, predominantly due to an increase in mesangial matrix, develops later although an increase in the matrix component of the mesangium can be detected as early as 5-7 years after the onset of diabetes (8-11). While GBM thickening may develop steadily over time, mesangial expansion has a more asymptotic relationship with T1DM duration (Steinke J, Mauer M, unpublished observations). However, when renal insufficiency occurs, marked mesangial expansion and increased GBM width are present in virtually all T1DM patients (9-10). Diffuse mesangial expansion, commonly termed diffuse diabetic glomerulosclerosis, can be associated with nodular lesions consisting of areas of marked mesangial expansion forming large round fibrillar mesangial zones with palisading of mesangial

nuclei around the periphery of the nodule and compression of the associated glomerular capillaries (Kimmelstiel-Wilson nodules). Both mesangial expansion and GBM and TBM thickening are a consequence of extracellular matrix (ECM) accumulation, with increased deposition of types IV and VI collagen, laminin and fibronectin (12-13). In contrast, initial interstitial expansion is primarily due to an increase in the cellular component of this renal compartment (14); increase in fibrillar collagen is measurable only in patients with advanced disease (14).

Afferent and efferent arteriolar hyalinosis may be present within a few years after diabetes onset (5) and this vascular lesion contributes to ischemic global glomerular sclerosis. Similar lesions may occur in the glomerular subendothelial space (hyaline caps) and along the parietal surface of Bowman's capsule (capsular drops).

Abnormalities of the glomerular-tubular junction (GTJA) occur as late manifestations of the disease (15) predominantly in patients with proteinuria, and rarely at earlier stages (16). These manifest with focal adhesions, obstruction of the proximal tubular take-off from the glomerulus detachment of the tubule from the glomerulus (atubular glomerulus). These focal segmental glomerulosclerosis (FSGS) lesions have a marked predilection for the GTJ and are uncommon at other locations. The lesions at the GTJ are inversely correlated with GFR (15, 16) and probably contribute to the loss of renal function in proteinuric diabetic patients.

These various lesions of diabetic nephropathy progress at varying rates within and between T1DM patients, and, as discussed below, this is even more the case in type 2 diabetes (T2DM). For example, GBM width and Vv (Mes/glom) are significantly but not very precisely correlated with one another; with some patients have relatively marked GBM thickening without much mesangial expansion and others the contrary (9). Marked renal extracellular basement membrane accumulation resulting in extreme mesangial expansion and GBM thickening are present in the vast majority of T1DM patients who develop overt diabetic nephropathy (DN) manifesting as proteinuria, hypertension, and declining GFR (8, 17). Ultimately, focal and global glomerulosclerosis, tubular atrophy, interstitial expansion and GTJA facilitate this downward spiral. However, tubulo-interstitial lesions and GTJA contribute only $\approx 10\text{-}15\%$ to functional loss in T1DM patients whose GFR is above $40 \text{ ml/min/1.73m}^2$ (16). Tubulo-interstitial disease may be more important in the progression from moderate renal insufficiency to end-stage renal disease (ESRD) (18), but it is probably a mistake to extrapolate this to earlier stages of DN progression. The situation in T2DM is more complex. The real frequency of non-diabetic renal diseases among patients with T2DM and proteinuria is difficult to assess in studies of which patients biopsied for clinical reasons because of selection bias towards atypical cases (19-24).

Research renal biopsies in a large cohort of T2DM patients with microalbuminuria (MA) and proteinuria and described marked heterogeneity in renal structure among these patients; in fact, only a minority subset had DN patterns typical of those seen in T1DM patients; the remaining had mild or absent diabetic glomerulopathy with or without tubulo-interstitial, arteriolar and global glomerulosclerosis changes (25). Less than 10% of our proteinuria patients had non-diabetic renal diseases. Based on these observations, we proposed a classification system which included 3 major categories (25):

Category C I: Normal or near normal renal structure. These patients (35% of MA and 15% of proteinuria) had normal renal biopsies or showed very mild glomerular, tubular, interstitial and/or vascular changes.

Category C II: Typical diabetic nephropathology. These patients (30% of MA and 50% of proteinuria) had established diabetic lesions with an approximately balanced severity of glomerular, tubulo-interstitial and arteriolar changes, a picture typical of that seen in most T1DM patients with obvious light microscopic DN changes.

Category C III: Atypical patterns of renal injury. These patients (35% of MA and proteinuria) had relatively mild diabetic glomerular changes considering disproportionately severe: (a) Tubular atrophy, TBM thickening and reduplication and interstitial fibrosis (tubulo-interstitial lesions). (b) Advanced glomerular arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels. (c) Global glomerular sclerosis. In C III group these patterns were present in all possible combinations. More recently, examining the associations of albumin excretion rates (AER) and electron microscopic morphometrically quantitated DN lesions, we could mathematically define a spatial cluster of structural/functional relationships which contained the T1DM patients. About 1/3 of the T2DM fell outside of this cluster because of MA or proteinuria despite a paucity of diabetic glomerulopathy lesions (26). These objective data largely confirm the more subjective categorical classifications.

Thus, hyperglycaemia may cause different patterns of renal injury in T1DM compared to T2DM patients. Alternatively, the disproportionate tubulo-interstitial, glomerulosclerotic and vascular changes of T2DM could also be related to aging, atherosclerosis and systemic hypertension. The natural history of MA and proteinuria T2DM patients with minimal or no renal lesions is not yet well understood, however, GFR loss in the relatively short-term (about 3 years), is largely confined to T2DM research patients with mesangial expansion (27).

Morphometric analysis and structural-functional relationships

The critical lesion in T1DM is mesangial expansion, morphometrically termed mesangial fractional volume [$V_v(\text{Mes}/\text{glom})$] (the fraction of the cross-sectional area of the glomerular tuft made up by mesangium); this is the electron microscopically estimated structural parameter that best correlates with all functional parameters in T1DM (9, 17). Indeed, a highly significant inverse correlation exists between $V_v(\text{Mes}/\text{glom})$ and GFR (9, 15-17); when mesangium expands it restricts and distorts glomerular capillaries and diminishes capillary filtration surface (9), which is strongly directly related to $V_v(\text{Mes}/\text{glom})$ and inversely to GFR (28). $V_v(\text{Mes}/\text{glom})$ is also related to AER (9, 15-17, 29) and blood pressure levels (30). In contrast, GBM thickening is closely related to AER and less so to GFR or hypertension, suggesting that this lesion is a closer surrogate to the pathogenesis of albuminuria. Interstitial expansion and percentage of global sclerosis are also directly related to proteinuria, hypertension and inversely to GFR (4, 5, 9, 15, 16). Progression from normoalbuminuria (NA) to MA and from MA to proteinuria is primarily related to progressive mesangial expansion (11) with no significant progression in interstitial fibrosis or GBM thickening over the 5 years of this study. These data may initially seem contradictory to recent studies describing that greater GBM width at baseline biopsy was predictive of AER after 5 or 6 years of follow-up (31, 32). However, given the linear course of GBM thickening *vs.* the non-linear trajectory of mesangial expansion, it is not surprising that GBM width, a strong correlate of AER, is a better predictor of DN risk while mesangial

expansion, through its intimate relationship with filtration surface, better defines the clinical course of those destined to develop severe diabetic kidney disease. Although an increase in AER to the MA range is usually considered the first clinical expression of DN, some long-term T1DM patients have reduced GFR as initial indicator of renal disease (33). This situation has also been seen in T2DM patients (34).

As alluded to above, through much of the natural history of DN lesions develop in complete clinical silence. When persistent MA and proteinuria supervene, lesions are often far advanced and loss of GFR may then progress relatively rapidly toward ESRD. This typical clinical story is best described by non-linear analyses of structural-functional relationships (16). Using simple linear regression models, glomerular structural variables explained about 65% of AER and 35% of GFR variability among T1DM patients (17). However, using piecewise (spline) regression models, glomerular structural variables alone, GBM width, [Vv(Mes/glom)], and total filtration surface per glomerulus or TFS], explained 95% of variability in AER ranging from NA to proteinuria. These same glomerular structures, however, explained only 78% of GFR variability in this study, and this increased to 92% with the addition of indices of GTJA and interstitial expansion (16).

In summary, most of the AER and GFR changes in T1DM are explained by diabetic glomerulopathy lesions and these structural-functional relationships are largely driven by patients with more advanced lesions and clinical functional abnormalities while structure is highly variable (from virtually none to moderate severity) in patients without functional abnormalities. In the end, as in other slowly progressive renal diseases, clinical findings in DN may, at least in part, reflect the lesions outstripping of renal compensatory capacities and this may be mirrored in the non-linear analyses described above.

Reversibility of diabetic nephropathy lesions

Pancreas transplantation offers the opportunity to test the effects of long-term normoglycemia to prevent, halt or reverse DN lesions. GBM and TBM widths were decreased after 10-years of normoglycemia, returning to normal values in most patients (35). Vv(Mes/glom) and mesangial matrix fractional volume [Vv(MM/glom)] were also lower at 10 years than at baseline or 5 years (35). Light microscopic observations revealed a remarkable amelioration of glomerular structure, including the total disappearance of Kimmelstiel-Wilson nodular lesions and reopening of glomerular capillaries previously compressed by mesangial expansion (35). These findings call for further studies aimed at identifying the molecular and cellular mechanisms involved in these healing processes which could provide new directions in the treatment of DN.

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