Published in final edited form as:

Life Sci. 2013 August 28; 93(7): 257–264. doi:10.1016/j.lfs.2013.06.016.

Associations between Structural and Functional Changes to the Kidney in Diabetic Humans and Mice

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Abstract

Type 1 and Type 2 diabetic patients are at high risk of developing diabetic nephropathy (DN). Renal functional decline is gradual and there is high variability between patients, though the reason for the variability is unknown. Enough diabetic patients progress to end stage renal disease to make diabetes the leading cause of renal failure. The first symptoms of DN do not appear for years or decades after the onset of diabetes. During and after the asymptomatic period structural changes develop in the diabetic kidney. Typically, but not always, the first symptom of DN is albuminuria. Loss of renal filtration rate develops later. This review examines the structural abnormalities of diabetic kidneys that are associated with and possibly the basis for advancing albuminuria and declining GFR. Mouse models of diabetes and genetic manipulations of these models have become central to research into mechanisms underlying DN. This article also looks at the value of these mouse models to understanding human DN as well as potential pitfalls in translating the mouse results to humans.

Overview and characteristics of diabetic nephropathy

Diabetic nephropathy (DN) is the largest single cause of end stage renal disease (ESRD). More than 25% of Type 1 and Type 2 diabetics will develop DN. Characteristics are similar in Type 1 and 2 diabetes (Tervaert et al., 2010, White and Bilous, 2000) and similar percentages of patients with both types of diabetes develop albuminuria and reduced glomerular filtration rate (GFR) (Jerums et al., 2009). Development of advanced DN is one of the single most important factors determining mortality for diabetics: In fact Type 1 diabetics without renal disease have mortality similar to that of nondiabetics (Orchard et al., 2010). This review describes research on renal structural changes associated with loss of normal kidney function and renal injury initiated by hyperglycemia. As increasing amounts of current research on DN is performed using mouse models, similarities and dissimilarities of human and mouse DN are highlighted throughout this review.

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Human DN develops slowly and inconsistently, requiring a few years in some patients but several decades in others (Figure 1A). Most often the first indicator is microalbuminuria. However the perception that microalbuminuria (de Zeeuw et al., 2004) is a certain predictor of future nephropathy has not been supported by longitudinal studies in Type 1 and 2 diabetics (reviewed in (Jerums, Panagiotopoulos, 2009, Retnakaran et al., 2006)). Many patients that develop microalbuminuria do not progress to macroalbuminuria or more severe DN. Microalbuminuria patients may even revert to normalalbuminuria (Jerums, Panagiotopoulos, 2009), and other diabetic patients with seriously impaired GFR never pass through a phase of albuminuria (Retnakaran, Cull, 2006). However, despite exceptions, urine albumin remains the best available indicator of future DN in diabetic patients.

Not only is there variability in the onset of DN, there is also variation in the rate at which renal function declines. The rate can be further changed by therapeutic intervention. Tight control of glycemia decreases the risk and slows the progression of DN in both Type I (DCCT, 1993) and Type II (UKPDS, 1998) diabetics. However, many diabetics with poor blood glucose control do not develop DN, while others, with good control progress to ESRD. Such patients are not rare and they demonstrate that factors other than glucose control are important to DN. Since there is a strong familial risk for DN (Thomas et al., 2012) inherited genetic variation is an obvious candidate. However there has been very limited success identifying genes in large patient populations that account for this inherited predisposition (Thomas, Groop, 2012). As with other complications of diabetes many culpable candidate factors have been proposed that can be influenced genetically or environmentally. These include oxidative stress (Ha et al., 2001), activation of protein kinase C (PKC) (Ha, Yu, 2001), advanced glycation end products (Vlassara and Palace, 2002) elevated sorbitol (Wallner et al., 2001) and increased TGF β activity (Ziyadeh, 2004). Probably several of these factors are involved, either through a sequential chain of events or by parallel pathways. The list of enzymes and compounds implicated in DN continues to expand, which mean that the full pathology is not yet understood and continues to grow.

The problem of dissecting the cause of DN is made more difficult by the complexity of renal cellular architecture. Glomeruli alone contain many specialized cells: Endothelial cells line the inside of the glomerular capillary network which receives blood from an afferent arteriole and drains blood from an efferent arteriole. Visceral epithelial cells (podocytes) form an interdigitating wrap surrounding the outside of the capillary network. Mesangial cells provide structural support to the capillary network and parietal epithelial cells cover the inner surface of Bowman's capsule. The different glomerular cells communicate with one another which is facilitated by their close proximity. Injury to one cell affects its neighbors. At some point in the long progression of DN each of these different cell types exhibit abnormalities. It is important to determine which changes are primary and which are secondary. Identification of the structural basis for declining renal function will add to our understanding of the mechanism of renal failure and provide anatomical markers that can be used to follow progression towards ESRD. This research will be accelerated by application of mouse models of DN combined with the ability to manipulate the mouse genome.

Glucose induced toxicity to the kidney

Hyperglycemia initiates, sustains and promotes the progression of DN pathology. Along with control of blood pressure and inhibition of the angiotensin system, careful maintenance of near normal blood glucose is the most effective means to delay the onset of DN (DCCT, 1993, UKPDS, 1998). Furthermore, kidneys with early to moderate DN may revert to almost normal structure and function if their glucose environment is greatly improved by transplantation of a healthy pancreas into a diabetic recipient (Fioretto et al., 1998) or a diabetic kidney into a non-diabetic recipient (Abouna et al., 1983). Improvement in renal

structure is a slow process as it is not apparent until ten years after a successful pancreas transplant (Fioretto, Steffes, 1998). Differences in blood glucose control are not sufficient to fully explain an individual's risk for developing DN. Other factors such as inflammation, hypoxia, hyperlipidemia may all play important roles. However each of these factors is initially triggered by high glucose. Therefore this section will limit its focus to direct toxic actions of glucose in the kidney.

High glucose produces damage inside and outside of the cell. Outside of the cell the major toxicity is from accelerated formation of advanced glycation end products (AGEs). These form when glucose or its metabolites produce stable, covalent modification of proteins. In the extra-cellular compartment, the major AGEs derive from direct reaction of glucose with protein amino groups. The modifications can be an isolated change to the peptide chain or multiple modifications that produce crosslinks within or between proteins. AGE modified proteins increase in several compartments of diabetic kidneys (Tanji et al., 2000). Long-lived extra-cellular proteins such as matrix proteins are particularly prone to accumulate AGE crosslinks (Susic et al., 2004). Multiple AGE crosslinks impair the degradation of extracellular matrix (Lubec and Pollak, 1980), tipping the balance towards accumulation of extracellular matrix and thickening of basement membranes. Circulating levels of AGE modified proteins also increase in diabetics (Penfold et al., 2010). These soluble AGE modified proteins are ligands for the RAGE receptor, which is increased on several cell types including podocytes of diabetic kidneys (Tanji, Markowitz, 2000). Activation of the RAGE receptor stimulates inflammation via the transcription factors NF- κ B and early growth response-1 factor (Ramasamy et al., 2011). Binding of the RAGE receptor also increases intracellular production of reactive oxygen species (ROS) via activation of NADPH oxidase (Goldin et al., 2006), which promotes intracellular damage.

Inside the cell elevated glucose accelerates several pathways shown in experimental models to alter and impair intracellular metabolism. The intracellular pathways were summarized in 2000 by Nishikawa et al (Nishikawa et al., 2000) and proposed as a general mechanism for all diabetic complications. This has been updated in a more recent review (Giacco and Brownlee, 2010). The proposal includes 5 pathways capable of producing cell damage or death: 1) The aldose reductase pathway, which can deplete intracellular NADPH. 2 and 3) The intracellular AGE and hexosamine pathways, which can modify proteins and thereby alter protein interactions and enzymatic activities. 4) The PKC pathway in which particular PKC isoforms are over-stimulated by excess diacylglycerol, a metabolite of glucose. 5) The mitochondrial superoxide pathway which generates excess superoxide if the mitochondrial membrane potential becomes hyperpolarized due to surplus electron donors generated by high rates of glucose metabolism.

Intracellular glucose toxicity requires that the high extracellular glucose enters the cell. Cells with insulin dependent glucose transporters, such as muscle cells have reduced intracellular movement of glucose in diabetes and they are thereby partially protected from glucose toxicity. Endothelial cells express glucose transporters that are independent of insulin, so they do not down-regulate glucose transport in the presence of high glucose and/or low insulin activity. This makes endothelial cells more vulnerable to high glucose toxicity. Different kidney cells express different members of the glucose transporter family (Heilig et al., 1995). In the glomerulus podocytes (Lennon et al., 2009) and mesangial (Heilig, Zaloga, 1995) cells express glucose transporters that are insulin dependent while endothelial cells express insulin independent isoforms. Wang et al (Wang et al., 2010) overexpressed glucose transporter 1 in mouse mesangial cells to increase glucose movement into these cells. Consistent with the concept that excess glucose transport sensitizes cells to glucose toxicity, glomeruli of these mice demonstrated many of the characteristics of DN even in the absence of diabetes. However when the same experiments were performed on podocytes (Zhang et

al., 2010) glucose transporter 1 overexpression did not produce injury and was even modestly protective from diabetes. With these apparently contradictory findings the role of glucose transport in DN is uncertain and may be cell type specific.

Onset and progression of structural damage

The structural pathology and functional deficits of the kidney are similar though not identical in Type 1 and Type 2 diabetic patients (White and Bilous, 2000). The similarity makes it possible to look at research results from the two types of diabetes together, while bearing in mind that they derive from different populations. For both types of diabetes excessive protein excretion and abnormal GFR are the major features of DN that are described herein.

Changes associated with albuminuria

In albuminuria there is a several fold increase in urine albumin above the extremely low levels of albumin present in the urine of healthy subjects. Albuminuria is usually stratified into microalbuminuria and macroalbuminuria. Different definitions of micro- and macroalbuminuria are used that depend on the method of urine collection, whether albumin content is normalized to urine creatinine concentration and what is the sex of the patient. Typically the onset of microalbuminuria occurs 5 to 15 years and macroalbuminuria 15 to 25 years (Adler et al., 2003, DCCT, 1995, Remuzzi et al., 2006) from the onset of diabetes (Figure 1A). Albumin is used as the marker of protein leakage because it by far the most abundant serum protein, making it easy to measure and because its size and charge prevent the vast majority of albumin from exiting a properly functioning glomerulus. The small amount of albumin that does escape a healthy glomerulus is captured by the brush border of proximal tubule cells and is thereby prevented from entering the urine. Megalin and cubilin are cooperating receptors in the proximal tubule brush border that bind proteins in the tubular lumen with moderate affinity and reabsorb these proteins (Birn and Christensen, 2006). The capacity of the system to reabsorb albumin is high. Once proteins are reabsorbed they enter the endocytic/lysosomal pathway where they are degraded and then reenter the circulation through the peritubular capillaries as amino acids or small peptides.

Albumin that enters the urine in diabetic patients could come either from many nephrons leaking a small amount of protein or it could come from a small number of nephrons that leak a large amount of protein. The scenario of a large number of leaky nephrons implies a relatively evenly distributed degree of functional and structural damage. The alternate scenario of a small number of severely leaky nephrons suggests a very uneven distribution of structural and functional damage to nephrons, including a large population of relatively normal nephrons and a small population of severely damaged nephrons. Whether examined by micropuncture, histological techniques or biochemical methods the two scenarios would produce a dramatically different picture. If damage to nephrons is relatively homogeneous then micropuncture, histological and biochemical results are likely to produce data that accurately represent the true condition of renal disease. On the contrary, if damage is markedly heterogeneous, then only a small number of very leaky nephrons may be located in the midst of a large population of near normal nephrons. Micropuncture would probably miss the few nephrons most important to albuminuria and abnormal biochemical results from the whole kidney or all glomeruli would dilute out results from the most damaged nephrons or glomeruli. Because it is difficult to link visible structural abnormalities to impaired function such as albumin leakage limited evidence is available to indicate how many nephrons or what specific characteristics contribute to albuminuria. Our laboratory used albumin that accumulated in proximal tubule cells as a marker for albumin leakage in human and mouse nephrons. OVE26 diabetic mice were studied because they have especially severe albuminuria (Kralik et al., 2009). In mouse and human diabetic samples

(Figures 2A–C) there was heterogeneity among different tubules with regard to albumin staining and presumably albumin leakage. In the diabetic mouse sample it appeared that the high level of albuminuria of this mouse came from a small number of nephrons (Figure 2A). From serial section studies it appeared that albumin stained diabetic mouse tubules always originated from glomeruli with adhesions between the tuft and Bowman's capsule (Kralik, Long, 2009). Adhesions have been proposed as the precursors to more extensive glomerular damage (Najafian and Mauer, 2012) and nephron loss (Kriz and LeHir, 2005). Because the proximal tubule can reabsorb a large amount of protein it is easier to get protein past the segment of tubular protein reuptake and into the urine if a large amount of protein leaks from a few glomeruli than if a small amount of protein leaks from many glomeruli. In humans the much longer proximal tubule presents a still greater obstacle to albumin entry into the urine than it does in the mouse.

The earliest structural abnormality that is routinely detected in diabetic kidney samples (Tervaert, Mooyaart, 2010) is glomerular basement membrane (GBM) thickening. This occurs in diabetic patients (Laviola et al., 2001, Steinke et al., 2005) and in diabetic mice (Qi et al., 2005) even in the absence of albuminuria. Thus initial GBM thickening appears to be more a result of abnormal diabetic metabolism, such as high glucose, rather than an indicator or consequence of albuminuria. However in patients that develop persistent microalbuminuria the GBM becomes still thicker, demonstrating that there is an association between protein leakage and progression of GBM thickening. Because the relationship between GBM thickness and albuminuria remains only an association it cannot be stated whether GBM thickening or albuminuria plays a role in causing one another. The same problem determining causality holds true for all of the other associations that exist between renal structural and functional abnormalities identified in diabetic patients.

Glomerular podocytes have a vital role in preventing albumin leakage into the urine. Mutations of podocyte genes, either achieved deliberately in mice (Putala et al., 2001) or naturally occurring in humans (Kestila et al., 1998), especially mutations in genes coding for slit diaphragm proteins result in profound proteinuria. The location of podocytes surrounding the GBM and glomerular capillaries place them in a strategic position to influence protein efflux from glomerular capillaries into the urinary space within Bowman's capsule. Minor structural damage to podocytes is indicated by widening of their attachment points to the GBM, referred to as effacement of podocyte foot processes. This initial podocyte damage can occur without development of microalbuminuria (Torbjornsdotter et al., 2005), indicating that podocyte foot process effacement is not sufficient for significant protein leakage. More serious damage to the podocyte can be visualized by electron microscopy as partial detachment of the podocyte from the GBM which tends to coincide with increasing albumin leakage (Toyoda et al., 2007). Further podocyte damage results in complete detachment of the podocyte from the GBM which can be ascertained by the presence of podocytes in the urine. Podocytes are recovered at low levels from urine of microalbuminuric patients and at higher levels from macroalbuminuric patients (Nakamura et al., 2000). Surprisingly, patients with end stage DN, presumably with high albuminuria and serious podocyte damage have almost no urinary podocytes (Nakamura, Ushiyama, 2000). Other studies that counted podocyte number (Steffes et al., 2001) and/or density (White et al., 2002) per glomerulus and compared this with the level of urine albumin demonstrated a significant negative correlation (White, Bilous, 2002); albuminuria goes up as podocyte number or density goes down. All of these results are in accordance with the essential role of podocytes in preventing protein leakage, a role that is compromised when there is significant damage to the podocyte beyond foot process effacement.

The structural basis for declining GFR?

Declining GFR with resultant azotemia causes morbidity and increasing mortality if patients do not die first from cardiovascular disease. Such severe DN occurs decades past the onset of diabetes (Adler, Stevens, 2003). The normal kidney has excess filtration capacity and individual nephrons are capable of increasing their filtration load to compensate for injury to other nephrons. So a great deal of filtration capacity must be lost before a clinically significant decline in GFR occurs. Kidney donors provide an obvious example of human reserve filtration capacity that allows donors to make up for a 50% loss of total nephrons without a rise in serum creatinine (Chen et al., 2012). Excess nephron capacity and compensation are consistent with the very late onset of clinically impaired GFR in DN (Adler, Stevens, 2003). Despite very extensive study of the pathology of reduced GFR the structural basis remains uncertain. A particular obstacle to this research is the virtual absence of mouse models of diabetes with severely reduced GFR. This is not surprising for a symptom of human DN that occurs decades beyond the life span of mice.

The glomerulus is the sole component of serum filtration, so the number or integrity of glomeruli is a prime determinant of GFR. Many structural abnormalities of the glomerulus correlate with declining GFR in diabetic patients: These include focal or global glomerulosclerosis, mesangial matrix expansion, and a decreased number of glomeruli. Glomerulosclerosis eliminates all or part of the glomerulus capable of filtration. Current opinion is that glomerulosclerosis begins with podocyte damage (D'Agati, 2012) in any region of the glomerulus. Local podocyte injury is proposed to damage adjacent podocytes, which may be the basis for the segmental appearance of early glomerulosclerosis (D'Agati, 2012) and may lead to expansion of sclerosis to the rest of the glomerulus. Regions of the GBM corresponding to areas where podocytes are lost become resurfaced with other cell types, probably parietal epithelial cells (D'Agati, 2012, Smeets et al., 2011). These are thought to be parietal epithelial cells because podocytes have little or no proliferative capacity and the replacement cells carry markers for parietal epithelial cells (Smeets, Kuppe, 2011).

Expansion of the glomerular mesangium reduces GFR by compressing glomerular capillaries thereby reducing filtration in portions of the glomerulus. This is further aggravated if large mesangial matrix nodules impinge on and occlude glomerular capillaries. Mesangial matrix formation rather than proliferation of mesangial cells corresponds most closely with declining GFR (Steffes et al., 1992). Growth of the mesangial matrix component of the mesangium is the main contributor to glomerular enlargement in Type 1 diabetic patients (White et al., 2007). The enlarged diabetic glomerulus shown in Figure 3 contains increased mesangium and fewer open capillaries than normal. The mesangial matrix expansion does not occur early in the process of DN. It is rarely detectable within 3 years after the onset of diabetes (Pagtalunan et al., 1997, Steffes, Bilous, 1992). Despite decades of glomerular damage and proteinuria the number of glomeruli per kidney does not decline in Type 1 diabetic patients with less than 20 years of diabetes (Bendtsen and Nyengaard, 1992).

Glomerular filtration capacity is completely eliminated if the glomerulus loses connection to the proximal tubule. This glomerulus is described as atubular. Glomeruli with abnormalities in the junction between the proximal tubule and the glomerulus may proceed towards irreversible formation of atubular glomeruli (Forbes et al., 2011, Najafian et al., 2006). The number of glomeruli with abnormal tubule junctions, which includes atubular glomeruli, correlates inversely with GFR (Najafian, Crosson, 2006, White et al., 2008). Atubular glomeruli are found in Type 1 (Najafian, Crosson, 2006) and 2 (White, Marshall, 2008) diabetic patients, though proteinuria appears to be a necessary factor for formation of atubular glomeruli only in Type 1 patients (Najafian, Crosson, 2006). Renal diseases

(Marcussen, 1992) or experimental manipulations (Forbes, Thornhill, 2011) that manifest primarily with tubulointerstitial fibrosis exhibit many atubular glomeruli leading to the hypothesis that interstitial fibrosis plays a role in damage to the proximal tubule/glomerular junction and ultimately formation of atubular glomeruli (White, Marshall, 2008). Interstitial fibrosis is the product of matrix formation by too many or over activated fibroblasts (Zeisberg and Neilson, 2010) as well as being the end product of renal scarring due to replacement of dead tubular, vascular and glomerular tissue (White, Marshall, 2008). In the diabetic sample in Figure 3 interstitial fibrosis is evident surrounding Bowman's capsule and between renal tubules. With few exceptions (Mohan et al., 2008, Zheng et al., 2011) interstitial fibrosis is one more feature of human advanced DN that is not found in mouse models of diabetes (Alpers and Hudkins, 2011, Brosius et al., 2009). In patient samples the degree of interstitial fibrosis (Okada et al., 2012) correlates with loss of renal clearance, this correlation is higher than the correlation of changes in renal function to damage to glomerular structure. Therefore interstitial damage has been proposed as a key determinant of progression of DN pathology. However, it has been pointed out that interstitial fibrosis occurs very late in DN and thus serves better as an endpoint rather than a cause or indicator of progression (Najafian and Mauer, 2012).

Utility of mouse models for DN research

Mice are vital to biomedical research due to almost unlimited changes that can be made to their genome. Mouse and human kidneys are similar in physiology, structure and cell types. Experimental diabetic mice maintain blood glucose levels (nonfasting 500–800 mg/dl (Liang et al., 2002, Susztak et al., 2004)) much higher than diabetic humans (nonfasting less than 350 mg/dl (Somannavar et al., 2009)). This greatly speeds the onset of DN (Figure 1) which accelerates the pace of research, even if it may modify pathology. In most lines of diabetic mice microalbuminuria or macroalbuminuria develop in weeks to months (Qi, Fujita, 2005, Susztak, Bottinger, 2004, Zhao et al., 2006, Zheng et al., 2004) but this requires many years in diabetic humans (Adler, Stevens, 2003, DCCT, 1995, Remuzzi, Benigni, 2006) (Figure 1). Mouse glomeruli demonstrate glomerulosclerosis, mesangial and glomerular expansion within months of diabetes onset (Brosius, Alpers, 2009, Qi, Fujita, 2005) rather than the years needed in human diabetics (Najafian and Mauer, 2012, osterby, 1974). Despite the acceleration of early DN, most mouse models fail to attain two key features of advanced DN: clinically significant reduction in GFR and extensive interstitial fibrosis. In humans these advanced features develop after decades (Adler, Stevens, 2003), so it is no surprise that they are not seen in the approximate 12 month post-diabetic lifespan of mice. In current diabetic mouse models research is generally limited to the early features of human DN. Since all cases of advanced DN must pass through early DN this is not an absolute deficiency.

Glomerulosclerosis and mesangial expansion occur in diabetic humans (osterby, 1974) and most mouse models (Zheng, Noonan, 2004) well before either species develop clinically significant reductions in GFR. These features of DN contribute to declining GFR but in early diabetes their effects on GFR are mitigated by diabetic hyperfiltration and compensation from healthy nephrons. This precludes measurement of abnormally low GFR early in the disease. However, quantitation of structural features of impaired filtration such as glomerulosclerosis and mesangial expansion could be considered quantifiable markers of progression towards advanced DN in diabetic mice and humans (Najafian and Mauer, 2012) during early DN.

Researchers have attempted to produce advanced DN in mice by further accelerating diabetic pathology. This improves the chances of attaining the endpoint of significantly reduced GFR, but increases the risk of achieving it through mechanisms not relevant to

human DN. The most successful advanced DN model has been the db/db eNOS mouse (Zhao, Wang, 2006), which displays extensive interstitial fibrosis and more than a 50% decline in GFR. This mouse is born with knockout of two genes, the leptin receptor and the eNOS gene. The db/db mouse is an established model of diabetes and DN (Wendt et al., 2003) carrying knockout of the leptin receptor that produces hyperphagia, insulin resistance and severe diabetes. The added knockout of the eNOS gene reduces nitric oxide production in many cell types, in particular endothelial cells. Both extreme hyperglycemia and reduced nitric oxide availability (Kietadisorn et al., 2012) of this mouse are exaggerated forms of known components of human diabetes. Blockade of the leptin pathway is not a normal component of human diabetes. Therefore it would be desirable to breed the eNOS knockout onto another diabetic mouse model and reproduce the phenotype of advanced DN.

The powerful research tools made available by manipulation of the mouse genome have generated an exponential rise in our knowledge of almost all aspects of renal embryology, physiology and pathology. Transgenic mice have enabled lineage tracing of most differentiated adult kidney cell types (Kobayashi et al., 2008). A goal to identify or produce multipotent renal stem cells capable of generating new nephrons following renal injury has not been reached, however more limited aims have been met. By genetically marking differentiated tubule cells (Lin et al., 2005) or renal progenitor cells (Humphreys et al., 2008) it has been shown that renal tubules contain cells capable of proliferating and replacing dead tubule cells. Extra-renal or extra-nephron stem cells are not the major source of replacement tubule cells and as discussed in the following paragraph, this also appears to be true of podocytes.

Since podocytes are essential to renal function but virtually incapable of proliferation, recovery from any disease producing a severe loss of podocytes requires a source of replacement podocytes. Over the past decade impressive evidence has evolved indicating that there is a regenerative program capable of replenishing disease depleted podocyte populations by transition of glomerular parietal epithelial cells (PECs) to a podocyte phenotype. An initial basis for the proposal was identification of peculiar cells on Bowman's capsule close to the glomerular, vascular pole, which contained both podocyte and PEC specific proteins that were not expected to be expressed in the in the same cell at the same time. This was observed in mouse (Appel et al., 2009), rat (Zhang et al., 2012) and human (Bariety et al., 2006, Smeets et al., 2009). Additional support came from EM identification of transitional cells located at the glomerular vascular pole that attach to both the GBM and Bowman's capsule, and possess a few simple foot processes (Appel, Kershaw, 2009). Transgene based lineage tracing, reported in the same paper (Appel, Kershaw, 2009) showed that podocytes can derive from PECs 5 days postnatal in the mouse. Adult human kidneys contain a subset of PECs that can differentiate into tubule cells or podocytes under appropriate cell culture conditions (Ronconi et al., 2009) or when injected into immune-deficient mice. These combined results provide substantial basis for the proposal that in adults PECs provide a reservoir of proliferating cells that can transform into differentiated podocytes. However, this does not preclude other proposed (Prodromidi et al., 2006) sources for podocyte replacement, such as bone marrow stem cells.

In renal disease PEC proliferation (Zhang, Hansen, 2012) and the number of PECs expressing podocyte proteins increase (Ohse et al., 2010) suggesting that the PEC based podocyte regenerative program is activated under conditions that injure podocytes. Injection of a human PEC sub-fraction expressing stem cell markers into Adriamycin induced nephrotic mice (Ronconi, Sagrinati, 2009) produces human podocytes and reduces nephropathy. The direct relevance of the podocyte regenerative program to diabetes has been presented for the BTBR ob/ob mouse model of severe insulin resistant Type 2 DN (Pichaiwong et al., 2013). In this model leptin replacement therapy reverses both diabetes

and DN, activates PECs and restores podocyte numbers. This suggests that PEC activation is a good target for therapy or prevention of DN. However, PEC proliferation and/or activation not only promotes podocyte replacement but it can also produce focal or segmental glomerulosclerosis (Smeets and Moeller, 2012). This danger will be an obstacle to overcome in therapy development.

Pathways of renal pathophysiology, expected and unexpected, have been revealed with transgenic mouse models. For example, glomerulosclerosis can be induced in transgenic mice by damage specific to the proximal tubule (Grgic et al., 2012). The role of reactive oxygen species in causing diabetic damage to podocytes has been proven in vivo with transgenic animals (Zheng et al., 2008). Homeostatic pathways of the podocyte regulated by insulin (Welsh et al., 2010) and mTOR (Godel et al., 2011, Inoki et al., 2011) are disrupted by diabetes and genetic manipulations of these pathways in mouse podocytes mimic DN. Much of the research described in the previous section on glucose induced toxicity to the kidney was performed with transgenic mice. Additional advances made with genetically modified mice are too numerous to mention.

Transgenic research into particular cells of the kidney requires promoters that express genes specifically in the cell type of interest. A useful list of kidney promoters has been published (Ly et al., 2011). Cells that can be targeted include podocytes, multiple different tubule cells, juxtaglomerular cells and recently parietal epithelial (Appel, Kershaw, 2009) cells. Unfortunately there are no promoters that allow transgenic targeting of two key cells of the glomerulus, mesangial cells and glomerular endothelial cells. Ample evidence implicates mesangial and glomerular endothelial cells in the pathology of DN, identifying new promoters specific for these cells will enable more specific experimental testing of their role in the pathology of DN.

Summary

DN is a major cause of diabetic mortality but there is a great deal we do not understand about its pathology. Some of these uncertainties are: The basis for very different patient susceptibility to DN despite similar blood glucose control. The cellular pathways of diabetes damage in glomeruli and tubules. The structural basis for albuminuria and declining GFR. By using mouse models of diabetes and genetic manipulation, research is being carried out faster and with greater precision. Now we need to confirm which results from mice apply to humans and those results need to be translated into improved therapies.

Acknowledgments

Supported by ADA grant 7-11-BS-37 and NIH grant DK072032 to PNE, JDRF grant 1-2011-588 to DWP and ADA grant 1-11-BA-17 to LC

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Figure 1. Approximate periods of normalbuminuria, microalbuminuria or macroalbuminuria in humans diabetes (A) or in mouse experimental models of diabetes (B)

The time scale on top is years from the onset of diabetes. The bars show the period of time after diabetes onset when more than 10% of diabetics exhibit normalbuminuria (open bars), microalbuminuria (dotted bars) or macroalbuminuria (solid bars). Figure A is derived from studies on Type 1 and 2 diabetes in humans (Adler, Stevens, 2003, DCCT, 1995, Remuzzi, Benigni, 2006). It should be noted that the majority of human diabetics do not develop DN in 20 years. Figure B is derived from studies of Type 1 and 2 models of diabetes in mice (Qi, Fujita, 2005, Susztak, Bottinger, 2004, Zhao, Wang, 2006, Zheng, Noonan, 2004). Essentially 100% of experimental diabetic mice develop early signs of DN within 2 months of diabetes.

Figure 2. Few tubules stain for albumin in mice and humans with diabetic nephropathy
Panel A shows a kidney section from an albuminuric OVE26 diabetic mouse stained by immunohistochemistry for mouse albumin (40X magnification). The arrow indicates the same albumin stained tubules which are magnified at 400X magnification in Panel B. Panel C is a needle biopsy sample stained for human albumin from a diabetic patient. Panels D–F are non-diabetic samples that correspond in species, staining and magnification to the diabetic sample shown in the panel immediately above.

Figure 3. Trichrome stained glomeruli obtained by needle biopsy from nondiabetic (A) and diabetic (B) patients

The diabetic glomerular tuft is enlarged due in part to expanded mesangial matrix. The diabetic sample demonstrates acellular nodular sclerosis (arrow) as well as periglomerular and interstitial fibrosis (**). Open capillary loops in the normal sample (*) are rare in the diabetic sample. Magnification 400X.