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The complex pathology of diabetic nephropathy in humans

Wilhelm Kriz[1], Jana Löwen[1] and Hermann-Josef Gröne[2,3]

[1]Department of Neuroanatomy, Medical Faculty Mannheim, University Heidelberg, [2]Medical Faculty Heidelberg, University of Heidelberg and [3]Institute of Pharmacology, University of Marburg, Germany

Correspondence to: Wilhelm Kriz; E-mail: wilhelm.kriz@urz.uni-heidelberg.de

ABSTRACT

This review summarizes the pathomorphological sequences of nephron loss in human diabetic nephropathy (DN). The relevant changes may be derived from two major derangements. First, a failure in the turnover of the glomerular basement membrane (GBM) based on an increased production of GBM components by podocytes and endothelial cells leading to the thickening of the GBM and accumulation of worn-out GBM in the mesangium. This failure may account for the direct pathway to glomerular compaction and sclerosis based on the continuous deposition of undegraded GBM material in the mesangium. Second, an increased leakiness together with an increased propensity of glomerular capillaries to proliferate leads to widespread plasma exudations. Detrimental are those that produce giant insudative spaces within Bowman's capsule, spreading around the entire glomerular circumference and along the glomerulo-tubular junction onto the tubule resulting in tubular obstruction and retroactively to glomerulosclerosis. Tubular atrophy and interstitial fibrosis develop secondarily by transfer of the glomerular damage onto the tubule. Interstitial fibrosis is locally initiated and apparently stimulated by degenerating tubular epithelia. This leads to a focal distribution of interstitial fibrosis and tubular atrophy accompanied by a varying interstitial mononuclear cell infiltration. Spreading of fibrotic areas between intact nephrons, much less to the glomerulus, has not been encountered.

Keywords: diabetic nephropathy, insudative lesions, mesangial matrix expansion, role of endothelial cells, role of podocytes, thickening of the GBM

BACKGROUND

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) in Western countries. Basic research concerning the pathophysiology of the renal disease is almost exclusively based on studies in rodents, mostly in mice, without taking sufficient care in the translation of the results to humans, as the human disease differs in essential morphologic aspects from those in mice.

Therefore, a review of the pathological changes of the human disease and their sequential progression to ESRD seems appropriate. This review tries to include the histopathological research of human DN from its beginning in the first half of the last century to the present time with an emphasis on phenomena that have not received adequate attention thus far. This review though does not pretend to cover and cite all publications that have indirectly or directly contributed to this integral assessment of DN.

Recent studies based on a large set of biopsies of DN from patients with type 1 and type 2 diabetes mellitus [1–3] have initiated this review. These studies are part of the basis for the present review as morphologic features have been partly reestablished, partly newly observed, and significantly extend currently accepted hallmarks of DN [4, 5]. A fresh look at the patho-morphology of DN may indeed be of help to get a grasp on its pathophysiology and to develop new concepts for prevention and therapy.

A note in advance. DN is a complex disease that may take years, even decades, to end up in ESRD. Apart from the initiating change of diffuse mesangial sclerosis (DMS) which underlies damage development in all cases, a large spectrum of various morphologies encountered in varying combinations characterizes DN. The review is structured into the four renal compartments and associated cells which form the fundamental and function determining phenomena of DN.

THE GLOMERULAR BASEMENT MEMBRANE AND MESANGIUM

The recognition of DN as a disease entity started with the description of nodular sclerosis in 1936 [6] followed in 1944 by DMS [7, 8]. The insight that the former develops from the latter [9, 10] has been a big step on the way to include the thickening of the glomerular basement membrane (GBM) into the trias of matrix expansion in DN [11, 12]. Since then this trias is considered as the defining structural derangement [13, 14]. Though the expanded mesangial matrix has been repeatedly termed GBM-like material [11, 12] even

including the observation that it contains "various abnormal structures" [15], it took almost half a century to realize the interdependence of mesangial matrix and GBM increase [1].

Even nowadays, the current opinion considers the thickening of the GBM and the expansion of the mesangial matrix to emerge independently from each other, namely that the thickened GBM with its complex components derives from podocytes and the increased mesangial matrix from mesangial cells [16].

In stark contrast to this dogmatic concept it has now been shown that the accumulated matrix in the mesangium for the most part consists of worn-out GBM material [1], stemming from the thickened GBM originally produced by podocytes [17, 18] and endothelial cells. We did not find any evidence for increased matrix production by mesangial cells contributing substantially to the expansion of the mesangium [1, 19, 20]. Moreover, in our original assessments [1] we found mesangiolysis in only 5% of all cases and concluded that it cannot be a specific feature of DN.

How does this contribution of the GBM to mesangial matrix expansion come about? The progressive thickening of the GBM leads to a narrowing and shortening of the spaces between capillaries and GBM infoldings followed by a cramping of podocytes within these clefts (Fig. 1A1 and A2). They retract out of these narrow spaces leaving behind shed cytoplasmic material which together with adjacent GBM portions protrudes like the ice plate of a glacier towards the mesangium and finally becomes entrapped in the mesangial matrix (Fig. 1B and C; depicted in schematics: Fig. 1D–F). The inclusions of podocyte remnants allow identification of these matrix portions as GBM material.

The thickening of the diabetic GBM and the subsequent mesangial deposition of these materials stems from an inadequate and elevated production of GBM components, among them the synthesis of α3 and α5 chains of collagen IV by podocytes (α3 chain shown in Fig. 2A) and of the α1 chain of collagen IV (Fig. 2B) by endothelial cells as proven by *in situ* hybridization (Fig. 2C). Perlecan, the production of which is normally restricted to glomerular endothelium of the embryonic kidney [21], is reactivated in endothelia in DN and can be shown in the increased mesangial matrix (Fig. 2D and D1).

The continuous deposition of worn-out GBM material in the mesangium takes center-stage in the progression of the disease. It presents as diffuse mesangial sclerosis. Thereafter, the depositions can lead to various phenomena. Accumulation in segments of the tuft leads to nodular sclerosis (Fig. 1C), frequently associated with a peripheral displacement of the nodules leading to contacts to Bowman's capsule (BC) (Fig. 3E). Predominant matrix accumulation in the vascular pole portion of the tuft causes the herniation of the tuft (Fig. 2E), which is generally associated with the outgrowth of glomerular capillaries through the vascular pole [2] (Fig. 2F). Ubiquitous progression over the entire tuft causes the retraction of podocytes out of the tuft, gathering on its surface (Fig. 2G, for details see below) and is followed by the degeneration of podocytes and parietal epithelial cells leading directly to global sclerosis [3] (Fig. 2H).

The mesangial matrix accumulation is sustained by the continued synthesis of GBM components by podocytes and endothelial cells. Whether a failure of mesangial cells to degrade the diabetic GBM contributes to the accumulation of GBM is speculative since the turnover of the GBM is insufficiently understood. In 1973, Walker [22] concluded from tracer studies that newly produced GBM components slowly move from peripheral positions to peri-mesangial positions, finally being incorporated into and degraded in the mesangium. Together with later studies using radio-labeled tracers [23, 24] the turnover of the GBM was calculated to be very slow (>100 days). In DN the synthesis of GBM compounds has been reported to be increased and the turnover prolonged [18, 25]. More recent studies about the precise turnover of the GBM in health and DN are not available. Moreover, despite a large body of knowledge about the matrix degrading capacity of mesangial cells [26, 27] we do not know which mechanism fails or supports the mesangial GBM accumulation in DN.

THE ROLE OF PODOCYTES

The essential role of podocytes in DN consists of the overproduction of GBM matrix. The question of whether podocytes are directly damaged by the hyperglycemic situation leading to podocyte loss is controversially discussed. From 1965 to 2021 many studies on DN have been done in rodent models of DN, mostly in mice, reporting that apoptosis of podocytes is an important mechanism for podocyte loss aggravating the progression of the disease. We know of only one study in biopsies of DN in humans claiming to have encountered apoptosis of podocytes [28], though without showing it by transmission electron-microscopy (TEM), the method by which apoptosis can be diagnosed without ambiguity. Podocytes in general seem to be protected against apoptosis. To our knowledge, in the entire literature only a single case of an apoptotic podocyte has been reported identified by TEM in a human biopsy (but not shown by a picture) [29]. In our studies [3], no evidence has been found that the diabetic condition causes a direct structurally recognizable damage to podocytes. In particular, we have never observed an apoptosis of a podocyte in our ultrastructural studies in several hundred biopsies.

Podocytes are lost in the course of DN, but not in early stages, which could be taken as evidence of a direct toxic effect of the metabolic situation to podocytes. This fully agrees with several previous studies that did not find a significant decrease of podocyte numbers in early stages of the disease [30–33].

Podocytes undergo cell death in advanced stages of the disease. These losses are secondary events due to preceding changes in the tuft. The continuous accumulation of worn-out GBM in the mesangium restricts the space for podocytes and capillaries. Capillaries collapse, podocytes either undergo cell lysis caused by mechanical stress or retract out of the GBM infoldings to position themselves on the tuft surface. Since they are largely segregated from any supply by capillaries including cross-talk stimuli from endothelia, they undergo regression and finally degenerate. These dying podocytes are

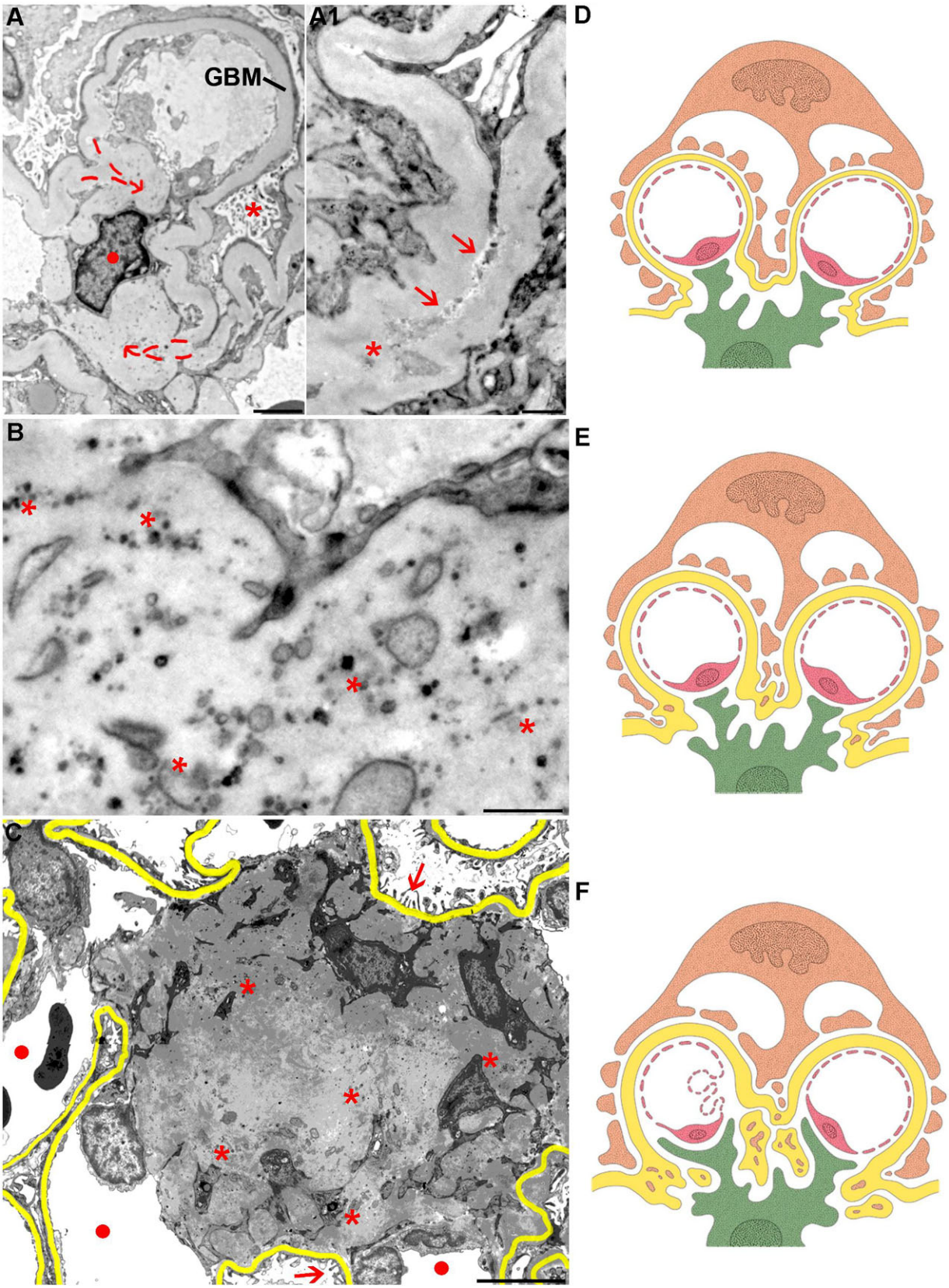

Figure 1: Matrix accumulation in the mesangium. (A–C) Transmission electron micrographs showing the incorporation of GBM portions into the mesangium. (**A**) The capillary loop is surrounded by a thickened basement membrane (GBM) that forms an infolding with a cleft (asterisk) that narrows toward the bend of the GBM, which merges (joining arrow) with a portion of matrix within the mesangium. A similar situation is seen on the opposite side also indicated by a joining arrow. Note the nucleus (dot) of a mesangial cell. (**A1**) An enlarged picture of an infolding of the thickened GBM with a narrow cleft. The cytoplasmic extensions of a podocyte are cramped, shedding cytoplasmic material into the cleft (arrows). At the innermost part of the infolding (asterisk) shed podocyte material becomes included into GBM material. (**B**) Mesangial area

Figure 1: (Continued) filled with matrix that contains randomly distributed cytoplasmic remnants of podocytes identifying the matrix as worn-out undegraded GBM material. (**C**) A sclerotic nodule bounded by the paramesangial GBM (highlighted in yellow) covered with structurally intact podocytes (arrows) and perfused capillaries (dots). In its peripheral parts it contains mesangial cells typically with many processes, but signs of any synthesizing activity are lacking. Throughout the nodule cytoplasmic remnants of podocytes (asterisks) are encountered, sparse in the very center, dense towards the periphery, allowing the conclusion that the accumulated matrix represents undegraded GBM-material. Biopsies DN. Scale bars: (A) 2.5 µm, (A1) 1 µm, (B) 0.5 µm, (C) 5 µm. (D–F) Schematics showing the sequence of GBM engulfment. The GBM is shown in yellow, a podocyte in pink, a mesangial cell in green and capillaries in red. (**D**) Normal situation. (**E**) Thickening of GBM, retraction of podocytes out of the constricted GBM infolding, leaving behind shed cytoplasmic material. (**F**) Dropping of the innermost portions of the GBM into the mesangium accumulating as mesangial matrix. The inclusions of shed cytoplasmic material from podocytes identify the matrix as undegraded GBM-material. (A–F) Reproduced from [1] with permission.

LC3 positive, however the classical signs of autophagy have not been encountered; the podocytes undergo cell lysis.

In cases in which a single lobule or only a part of it is afflicted, retracting podocytes assemble in clusters in Bowman's space and either undergo cell lysis or are washed away with filtrate flow [3].

When this process of podocyte retraction encompasses the entire glomerular convolute, podocytes congregate as a corona on the tuft surface (Fig. 2G). Due to the collapse of Bowman's space, they are broadly pushed against the parietal epithelium. Finally, both epithelial cell layers degenerate and global tuft sclerosis ensues (Fig. 2H).

THE ROLE OF ENDOTHELIAL CELLS AND GLOMERULAR CAPILLARIES

In diabetes, injuries of the microvasculature are found at many sites in the body. In DN, in addition to the important contribution of endothelial cells to the abnormal production of GBM components, two additional defects are relevant. First, an increased endothelial leakiness and second, an increased propensity of capillaries to proliferate.

The increased leakiness of glomerular capillaries is probably based on a defect in the endothelial glycocalyx, first described four decades ago [34]. It is directly caused by the hyperglycemia-induced metabolic derangements and widely considered as the cause of microalbuminuria in early stages of the disease [34–36]. Moreover, it likely accounts for the extensive insudative lesions at various sites in DN [5, 37].

Hyalinosis of glomerular arterioles is a well-known phenomenon in DN elicited by the exudation of a plasma exudate into the vessel wall. Hyalinosis of glomerular capillaries starts with a separation of the endothelium from the GBM and the accumulation of the hyaline material within the GBM bounded contours of the former capillary. Extensive hyalinosis of glomerular capillaries may contribute to direct global glomerulosclerosis by matrix accumulation in the mesangium (Fig. 2H).

Significant plasma exudations are seen in conjunction with the outgrowth of glomerular capillaries from the glomerular tuft. This capillary outgrowth takes place at two sites, at the vascular pole together with herniation of the tuft (as already described) and in conjunction with the formation of tuft adhesions to BC.

The outgrowth of capillaries from the tuft at the vascular pole in DN (Fig. 2F) was first described by Min and Yamanaka [38] and Osterby and colleagues [39]. Increased levels of vascular endothelial growth factor (VEGF) have been suggested to underlie the abnormal proliferation [40, 41]. We, in addition, have found significantly higher mRNA levels of angiogenic factors (VEGFA, Ang1 and Ang2) and their receptors (VEGFR and Tie2) in glomerular cells of patients with DN compared with controls [2].

The phenomenon of outgrowing vessels through the glomerular entrance has been encountered in more than 50% of biopsies [2]. Both, the herniation of the tuft and the outgrowth of vessels destroy the specific topography of the juxtaglomerular apparatus. The macula densa loses its contacts to the extraglomerular mesangium and to glomerular arterioles (Fig. 2E and F). Thus, a regulation of glomerular filtration by the tubulo-glomerular feedback seems improbable at this stage.

The majority of the outgrowing vessels are arteriole-like with depositions of hyaline material in their walls as frequently seen in glomerular afferent and efferent arterioles. A minor fraction are capillaries. These aberrant vessels penetrate into the interstitium between periglomerular tubules (Fig. 2F) and may drain into peritubular capillaries. Moreover, they frequently extend alongside the BC around the entire glomerular circumference toward the urinary pole [2]. The herniation of the glomerular capillary convolute through a widened vascular pole may even be of help in differentiating other diseases that may also manifest themselves by diffuse or nodular mesangial sclerosis such as light chain deposit disease, fibrillary glomerulopathy or idiopathic nodular glomerulosclerosis in patients with nicotine abuse.

Moreover, and quite specific for DN, outgrowths of glomerular capillaries are regularly seen in conjunction with formation of tuft adhesions to BC (depicted in schematics in Fig. 3A–D). Local accumulations of matrix (e.g. nodules) lead to peripheral displacements of capillaries resulting in contacts of structurally intact podocytes to the parietal epithelium (Fig. 3E). Such contacts may occur at any site of the glomerular circumference starting the formation of tuft adhesions to BC. Capillaries associated with the adhesions (Fig. 3E) penetrate through the GBM (Fig. 3F) leaving their basement membrane, the GBM, behind and spread within BC inside the parietal basement membrane (Fig. 3G and H). This process was first shown in an electron micrograph by Osterby in 1987 [42].

These capillaries deliver an exudate into the space between parietal epithelium and its basement membrane (PBM). Favored by the increased leakiness and the high perfusion pressure, giant insudative spaces within BC are formed that may spread around the entire glomerular circumference and

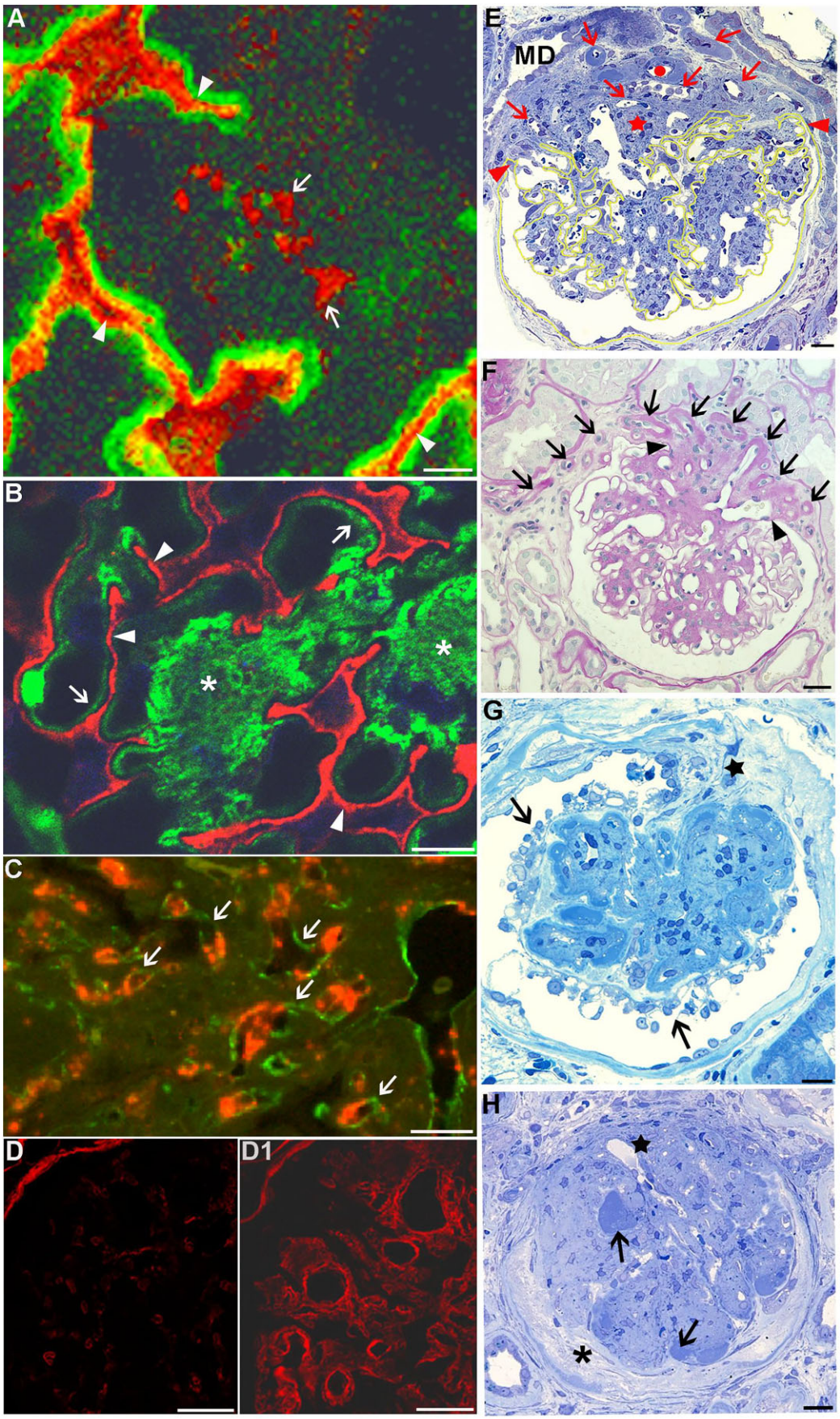

Figure 2: Immunofluorescence (IF) data identifying the origin of the accumulated matrix in the mesangium. (**A**) IF double staining against the α5 chain of collagen IV (green) and synaptopodin (red). Podocytes are cramped in narrow clefts of the GBM infoldings (arrowheads). Displacements of podocyte particles stained in red (arrows) into the mesangium surrounded by diffusely green staining GBM material (α5 chain). (**B**) IF double staining against the α1 chain of collagen IV (green) and synaptopodin (red). Note the cramped podocytes within the GBM clefts (arrowheads), and the intense staining of the GBM (arrows) and of the mesangial matrix (asterisks). (**C**) *In situ* hybridization (ISH) of the α1 chain of collagen IV (red) combined with IF localization of CD31 (green) clearly showing that the mRNA signals (red) are found only in endothelial cells (arrows). (D and D1) IF staining for perlecan. (**D**) Control, virtually no staining for perlecan in the heathy glomerulus. (**D1**) Biopsy DN. The GBM is stained throughout the tuft and a faint staining is seen in mesangial areas. Biopsies DN. Bars: (A) 5 µm, (B) 10 µm, (C) 20 µm, (D and D1) 40 µm. (A, C and D) Reproduced from [1] with permission; (B) original. Matrix propagation changes in overall tuft structure. (**E**) Overview of a glomerular profile with herniation of the tuft to the outside. The GBM and the parietal basement membrane PBM are highlighted in yellow. Note that the transitions between both (arrowheads) are displaced sideways showing that the upper portion of the tuft (star) has come to lie outside of the borders of the glomerulus. This herniated area is devoid of podocytes. It contains the efferent arteriole (dot) and several outgrowing vessels (arrows), some of them with hyaline cuffs. Apart from macula densa (MD) an assembly of structures that may be called JGA cannot be delineated. The rest of the tuft shows segmental sclerosis. (**F**) Glomerular profile with an extremely widened glomerular entrance delimited by the transition of the GBM into the PBM (marked by arrowheads). Thus, the upper portion of the tuft is herniated to the outside with outgrowth of a bunch of vessels (arrows; all of them with hyaline cuffs) occupying the area of the former JGA. Some of the vessels penetrate in between adjacent tubules. The rest of the tuft shows diffuse to segmental sclerosis. (**G**) The compacted tuft is largely filled with matrix (asterisks). Podocytes have retracted out of the tuft gathering on its surface (arrows). They are heavily distorted (LC3 and beclin-1 positive; not shown). The vascular pole is marked by a star. (**H**) In addition to solid matrix, the global sclerotic tuft contains several hyalinotic areas (arrows). Podocytes and parietal epithelial cells have disappeared, Bowman's space is filled with a fibrous matrix (asterisks) thus damage progresses to global glomeruosclerosis. Biopsies DN. All images are light micrographs. (E, G and H) Stained with methylene blue, (F) with PAS. Bars: (E, G and H) 20 µm, (F) 30 µm. (E and F) Reproduced from [2] with permission; (G) reproduced from [3] with permission; (H) original.

via the glomerulo-tubular junction onto the tubule (Figs 3G, H and 4A). The plasma exudate contained in the insudative spaces has previously been considered as hyaline material (hyaline drops) [5].

This kind of plasma exudation is different from previously published forms of "misdirected filtration" in rat models of focal segmental glomerulosclerosis [43], in which glomerular capillaries together with their GBM cover spread within BC. Here, in human DN, the capillaries penetrate through the GBM delivering their exudate just through a leaky endothelium devoid of a basement membrane.

The encroachment of these plasma exudations onto the tubule causes obstruction of the tubule (Fig. 4A) followed by cessation of filtration and collapse of Bowman's space, finally leading to glomerular decay. This sequence based on the spreading of a plasma exudate within BC and upon the tubule represents the most frequent pathway to global glomerulosclerosis in DN.

THE TUBULO-INTERSTITIAL DAMAGE

Glomerulosclerosis and tubular atrophy with interstitial fibrosis—so-called IFTA—mark the final stage of nephron dropout in diabetic nephropathy (Fig. 4B). Interstitial fibrosis, characterized by a monocyte/macrophage influx and by myofibroblast proliferation and matrix deposition, is locally initiated and maintained by paracrine stimulation from the degenerating tubular epithelia. The mediators are insufficiently known; transforming growth factor-β seems to play a central role [44].

Macrophages and lymphocytes can be seen in varying densities in these areas of IFTA, frequently considered as inflammatory infiltrations. The interstitial immune cells are exclusively found associated with degenerating tubules (Fig. 4B). They represent a reaction to the degenerating tubular process and are by no means a specific phenomenon of DN. Of note, structurally intact nephrons are generally found in their immediate vicinity (Fig. 4B) or even as single tubular profiles fully surrounded by degenerating tubules and proliferating interstitial tissue (Fig. 4C). No evidence has been found that such foci may have a progressing potential in itself. In our view, the final fibrotic process represents a healing process, which replaces degenerated tubules and glomeruli resulting in the formation of a scar.

The focal distribution of the tubulo-interstitial changes strictly correlated with the focal distribution of the glomerular changes [3], which is highly suggestive that they depend on each other. There are two ways in which the glomerular damage encroaches upon the tubule. First, in cases in which the accumulation of the undegraded GBM in the mesangium directly advances to global sclerosis (Fig. 2H), filtration gradually ceases; the corresponding tubules are deprived of any filtrate load and go into tubular atrophy, and later atresia [3]. This agrees with previous studies under different circumstances [45, 46]. A second pathway leading to tubular atrophy consists of the spreading of plasma exudates from capillaries within BC via the glomerulo-tubular junction onto the tubule. The epithelium of the proximal tubular convolution dissociates from its basement membrane, atrophies (Fig. 3H) and finally decays irreversibly. The tubular basement membrane or its remnants survive for a longer time and are likely removed by phagocytic macrophages. No evidence can be found in the literature or in our studies that tubular or tubulo-interstitial damage occurred without any damage in its respective glomerulus. In an analysis of 162 glomeruli, in which the glomerulo-tubular junction could be assessed, tubulointerstitial damage was exclusively found (87 cases) when the impingement of the glomerular damage onto the tubulo-interstitium was clearly seen; in cases with severe glomerular damage but without signs of encroachment (75 cases) the tubulo-interstitium was intact [3]. Thus, and in agreement with previous assessments in DN [4, 12], tubular atrophy and fibrosis were unequivocally

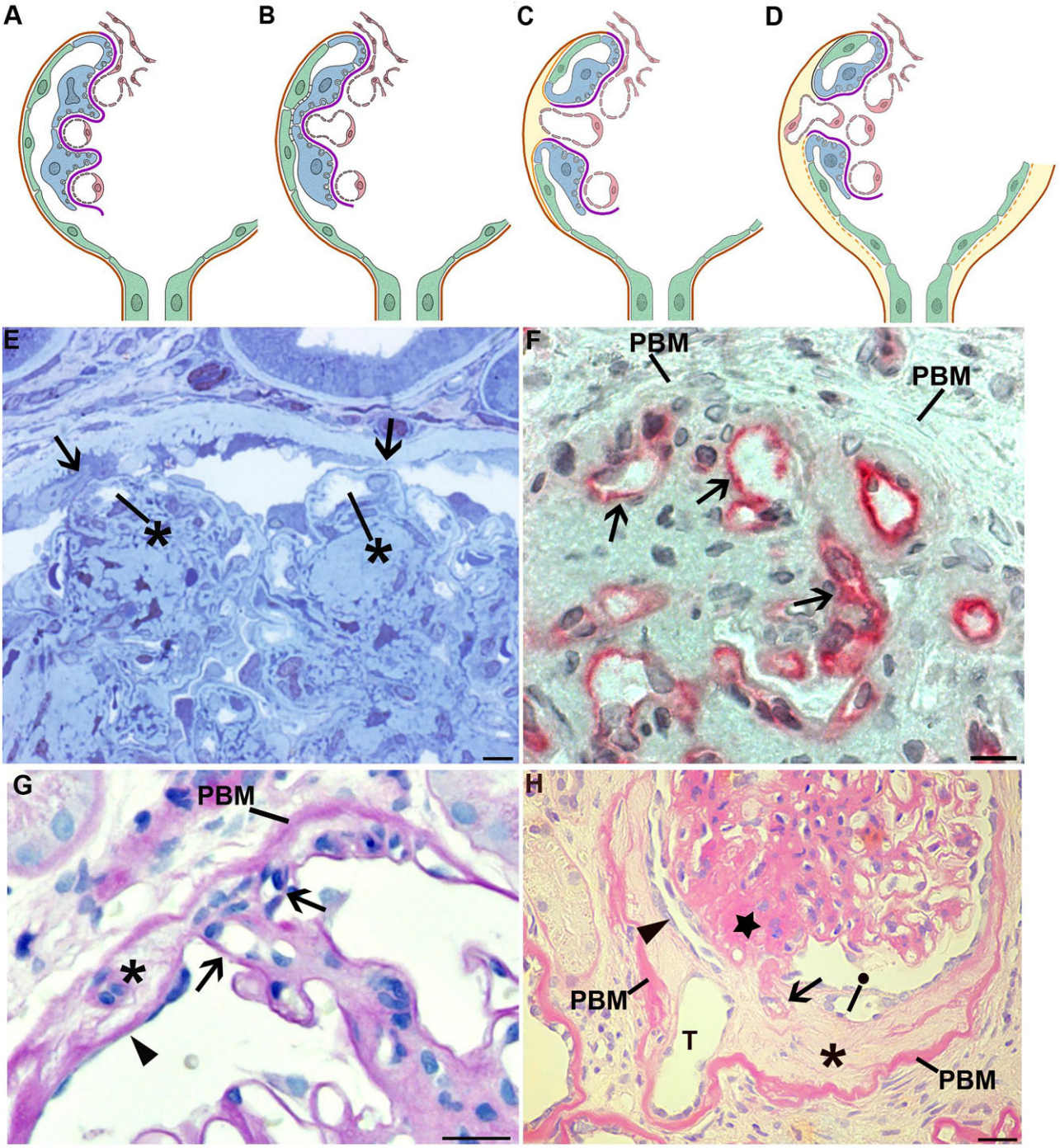

Figure 3: Formation of tuft adhesions and spreading of insudative changes. (A–D) Schematics. (**A**) Normal situation. Podocytes (shown in blue) are sitting on the GBM (shown in violet) that covers three capillaries (shown in red). Parietal epithelial cells (PECs) are shown in green. The PBM is shown in brown. (**B**) Local expansion of the tuft leads to contacts of podocytes to PECs. (**C**) The junctional contact of podocytes to PECs results in a gap in both epithelial layers followed by a gap in the GBM. Podocytes extend on the PBM. A glomerular capillary deprived of the GBM delivers an exudate into the space between the parietal epithelium and its basement membrane (PBM). PECs produce a new basement membrane (dashed in pale brown). (**D**) The exudate spreads within BC circumferentially around the glomerulus and, via the glomerulo-tubular junction, onto the tubule separating the tubular epithelium from its basement membrane (TBM). (E–H) Selected originals. (**E**) Due to matrix accumulations displaced perfused capillaries (asterisks) lead to contact sites of podocytes with the parietal epithelium (arrows). (**F**) CD31-stained glomerular capillaries that have penetrated into BC and have come to lie inside the PBM. (**G**) Early adhesion with two capillaries (arrows) penetrating into BC became to lie inside the PBM. Between the PBM and the parietal epithelium (that has produced a thin new basement membrane) a fluid-filled space has developed (asterisk). (**H**) Tuft adhesion (star) with outgrowth of a glomerular capillary (arrow) into a fluid filled space (insudative space) within BC that is bordered inside by the parietal epithelium (arrowhead), outside by the PBM and, in continuation, by the TBM. The beginning portion of the tubule (T) is atrophied, deprived of its basement membrane and included into the fluid-filled space (asterisk), as well as a further capillary (dot). Biopsies DN. (E–H) LMs, (E) stained with methylene blue, (F) immune-stained with CD31, (G and H) stained with PAS. Bars: (E and H) 10 µm, (F and G) 20 µm. (A–D and F–H) Reproduced from [3] with permission; (E) original.

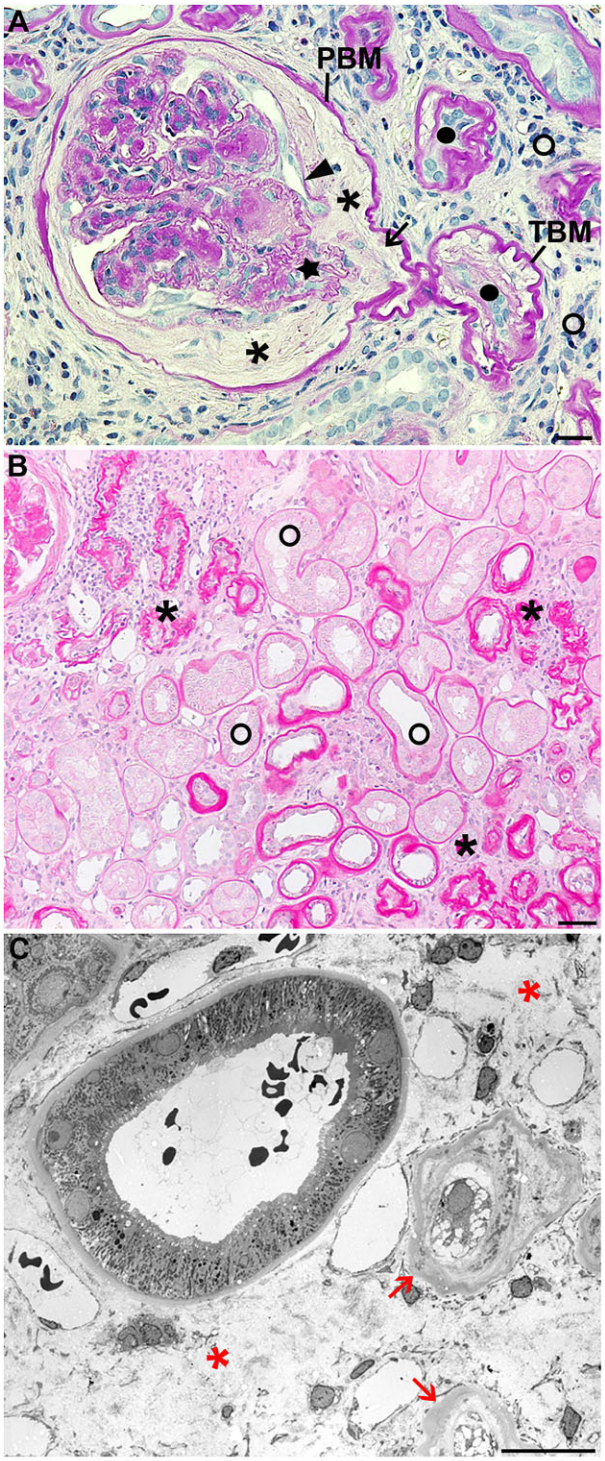

Figure 4: Tubulo-interstitial changes. (**A**) Tubulo-interstitial changes develop exclusively by transfer from the glomerulus. Shown here is the encroachment of the insudative changes in BC onto the tubule. Within BC the parietal epithelium (arrowhead) has been separated from its basement membrane (PBM) by plasma exudation from outgrown capillaries. The resulting insudative spaces within BC spread around the entire glomerular circumference. Via the glomerulo-tubular junction they extend (arrow) onto the tubule separating the tubular epithelium from its basement membrane (TBM); the tubular epithelium undergoes degeneration (dots) leading to obstruction. This process extends to subsequent tubular coils. Finally, wrinkled remnants of the TBM surrounded by

Figure 4: (Continued) proliferating interstitial tissue (circle) are left. Note, the starting point of this development, the original adhesion of the tuft to BC (star) is still seen. (**B**) Tubulointerstitial area with intermingled damaged (asterisks) and intact tubules (open circles). No tubular profile can be seen that displays intact and injured portions. No signs of a damage encroachment from damaged to intact tubules is encountered. The degenerating group of tubules in the upper right corner is surrounded by proliferating interstitial tissue. (**C**) Profile of a proximal tubule with a structurally healthy epithelium. This intact tubule is fully surrounded by fibrotic tissue with degenerated tubular profiles (_). Proximal tubule with a structurally healthy epithelium. This intact tubule is embedded in fibrotic tissue (asterisks) that contains also degenerated tubular profiles (arrows). Biopsies DN. (A and B) LMs stained with PAS, (C) is a transmission electron micrograph. Bars: (A and C) 20 µm, (B) 50 µm. (A–C) Reproduced from [3] with permission.

dependent in their initiation on the fibrosing and exudative angiogenetic processes of the glomerulus. This conclusion is in agreement with experimental findings in other glomerular diseases [46, 47].

These data stand in stark contrast to hypotheses claiming that the damaging processes in DN may also start from a primary tubular injury initiated by the metabolic situation [48] or by local hypoxia [49]. Without any question tubules are directly affected by the hyperglycemic situation. Already in early stages of the disease, the tubular basement membrane (TBM) undergoes a thickening [14, 50], as do the GBM and the glomerular PBM. Tubular hypertrophy, increased re-absorptive labor and overproduction of reactive oxygen species in DN have been shown to dispose tubular epithelia to increased cytokine production which may lead to pro-inflammatory and pro-fibrotic signaling under stress [48, 51].

The postulate that such changes may independently of any glomerular contribution progress by themselves to tubular atrophy and fibrosis is not supported by morphologic evidence. The thickening of the TBM may well interfere with the re-absorptive and secretory functions as well as the oxygen availability of the tubular epithelium, but no evidence has been provided that this can lead to any dedifferentiating damage of the tubular epithelium or changes in the peritubular interstitium. However, it is known that the diabetic tubular epithelium is vulnerable to potentially toxic substances (radiocontrast agents [52]), especially in states of dehydration and reduced tubular filtrate load. An increased vulnerability has also been found to SARS-CoV-2 infections [53].

HISTOPATHOLOGY AND THERAPEUTIC OPTIONS

The presently available therapeutic options of DN have been recently summarized [54]. Comparing them with the targets presented in this review shows reasonable agreements but also options for further rational approaches.

The defects of the glomerular microcirculation offer therapeutic targets with respect to glomerular hypertension and hyperfiltration, to the increased endothelial leakiness and to the increased propensity of glomerular capillaries to proliferate which may be encountered separately or in conjunction.

Table 1: Summary: the relevant phenomena are summarized in table form.

Podocytes
Overproduction of GBM components (e.g. α3, α4 and α5 chains of collagen IV) leads to thickening of the GBM. The surplus matrix cannot be degraded and is accumulated in the mesangium.
Direct podocyte loss initiated by the metabolic situation does not occur.
Secondary podocyte loss occurs due to environmental constraints including matrix deposition in the mesangium and collapse of capillaries.
Displaced, but intact podocytes start the formation of tuft adhesions to BC.
Endothelial cells and capillaries
Overproduction of GBM components (e.g. α1 and likely α2 chains of collagen IV and Perlecan) contributes to the thickening of the GBM. The surplus matrix cannot be degraded and is accumulated in the mesangium.
Increased propensity of capillaries to proliferate leads to outgrowth of glomerular capillaries through the vascular pole (together with herniation of the tuft) and to the outgrowth into BC in conjunction with the formation of tuft adhesions.
Increased endothelial leakiness due to damage of the glycocalyx has multiple effects.
First, it is responsible for the microalbuminuria in early stages of DN.
Second (likely together with the increased blood pressure), it causes widespread plasma exudations leading to hyalinosis of glomerular arterioles, glomerular capillaries and to outgrowing vessels through the glomerular vascular pole.
Most important, plasma exudation from capillaries in tuft adhesions results in fluid accumulations within the BC, which encroach upon the tubule leading to nephron dropout.
Mesangial cells and mesangium
No essential active contribution to disease development and progression. Whether a failure in the degradation of matrix may contribute to matrix accumulation is unresolved.
Mesangiolysis was rarely encountered and does not seem to be a specific injury of DN.
Accumulation of matrix in the mesangium represents one of the essential changes in DN. It is based on the ongoing deposition of worn-out GBM matrix. It leads to DMS, nodular sclerosis, herniation of the tuft and, in a fraction of cases, directly to global tuft sclerosis.
Parietal epithelial cells
Production of a thickened PBM.
Undergo degeneration after collapse of Bowman's space.
Tubular epithelial cells
Production of a thickened TBM.
Undergo atrophy, finally degeneration either when deprived from any filtrate or upon obstruction by a plasma-derived fluid encroaching from the BC.
Degenerating tubular cells release fibrogenic stimuli.
Interstitial fibroblasts and interstitium
Fibroblasts develop into myofibroblasts upon stimulation from degenerating tubular epithelia.
Production of matrix around degenerating tubules presenting as local areas of fibrosis with influx of monocytes/macrophages.
Spreading of fibrotic areas into intact tubulo-interstitial areas, much less to the glomerulus, has not been encountered.

Decreasing the exudation of plasma through the leaky endothelia of glomerular vessels represents a main target. These leakages account for albuminuria in early stages of DN, the widespread hyalinosis and, most important, the formation and extensive spreading of a plasma-derived fluid within BC and onto the tubule. The latter mechanism is predominantly responsible for the final loss of nephrons.

Thus, damping of glomerular hypertension and hyperfiltration [55] rightly represents a powerful measure. Angiotensin blockade at an enzymatic or receptor level has been established to suppress these two deleterious components. In addition to angiotensin II–blocking measures, decreasing sodium–glucose reabsorption by inhibition of sodium–glucose co-transporter-2 (SGLT2) is specifically considered to stop hyperfiltration via a feedback mechanism involving the juxtaglomerular apparatus (JGA) [51]. A similar mechanism has been proposed for glucagon-like peptide 1 (GLP-1) receptor agonists and dipeptiyl-peptidase-4 (DPP-4) inhibitors [56].

Since in stages of the disease with herniation of the tuft through the glomerular vascular pole the intimate topographical relationships of the JGA are lost, such a mechanism may only be effective in early stages of the disease. In late stages the beneficial effects of SGLT2 inhibition seem to be based first on the increased excretion of glucose and sodium and second, on an elevation of the hydrostatic pressure within Bowman's space due to increased tubular flow resistance resulting in a fall of hyperfiltration [57].

Surprisingly SGLT2 inhibitors have beneficial effects independent of their inhibition of glucose reabsorption in the proximal tubule, which are based on alleviating the dysfunction of microvascular endothelia by improving mitochondrial activity, decreasing reactive oxygen synthesis and by suppression of angiogenesis. The latter effect has now been convincingly shown [58]. The stymieing of the increased propensity of glomerular capillaries to proliferate would seem a relevant (powerful) option to decelerate progression of DN. As shown above, VEGFA contributes to the abnormal propensity of glomerular vessels to proliferate. Inhibition of the increased VEGFA production in DN have so far been frustrating, with severe adverse effects [59] including glomerular thrombotic microangiopathy [60, 61]. However potential new approaches may be feasible [62]. Recent experimental studies [63] have described a reduction of podocytic VEGF by SLGT2 inhibition. The same holds true for approaches to corrugate the defects in the endothelial glycocalyx [64, 65] by antagonism to endothelin and consequent heparanase action on the glycocalyx; these options have been uncovered experimentally but therapeutic solutions in humans are missing.

Even worse, no therapeutic options are currently available to decrease the overproduction of GBM components by

podocytes and endothelial cells; tight control of the metabolic situation represents the only measure to stabilize the situation.

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DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

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