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Review Article

Krzysztof Okoń

Tubulo-Interstitial Lesions in Glomerulopathy. I. Pathogenesis

Department of Pathomorphology, Collegium Medicum, Jagiellonian University, Kraków

Glomerulopathies (GLN) are at the focus of interest of nephropathologist. As the name implies, the glomeruli are most characteristically affected. Beside glomeruli, also tubules and interstitium are important participants in renal scarring. It is believed that its chief determinant is proteinuria. The presence of protein in the urine activates tubular epithelial cells and induces production of mediators, the most important being TGF-β. Subsequently, the interstitial cells are activated and acquire the myofibroblast phenotype. The myofibroblasts produce and deposit connective tissue matrix, resulting in morphologically visible scarring.

Medical kidney diseases are usually divided into vascular nephropathies, tubulo-interstitial nephropathies and glomerulopathies or glomerulonephritis (GLN). The terminology may suggest that in the case of glomerulopathy, the glomerulus is the only involved component of the renal parenchyma. This is not the case; moreover, processes that occur outside the glomerulus as such are of a great significance for the course of the disease and the prognosis. Glomerulopathies are less common than tubulo-interstitial or vascular nephropathies. At the same time, glomerulopathy is the starting point in renal failure in as many as 30 - 45% of dialyzed patients and individuals receiving renal transplantation. The incidence of chronic renal failure developing in the course of GLN is assessed to 12 - 27%. The progression to renal failure in patients with glomerular diseases is both faster and less predictable than in the case of other chronic renal diseases. Hence the need to develop methods that would allow for establishing a more precise prognosis. Such methods would have to be based on the knowledge of the mechanisms governing the pathogenesis of renal failure development [4, 23, 66, 102].

The pathogenesis of extraglomerular changes is complex and only partially explained, but the following mechanisms are postulated [13, 27, 28, 46, 47, 53, 62]:

- a transfer of cytokines and other mediators from the glomerulus to the lumen of the tubule and the interstitium,
- a damaging effect of excess of proteins on the tubular epithelium,
- an impaired blood and oxygen supply *via* a decreased diameter of the extraglomerular capillary vessels [7, 38, 39, 40, 51],
- a metabolic overload of the intact nephrons,
- a deposition of immunoglobulins or immune complexes within the tubules,
- cellular-type reactions, including a transfer of effector cells from the mesangium to the interstitium [11, 43, 47, 60],
- an interrupted integrity of the tubule, which may result in the Tamm-Horsfall protein penetrating the interstitium and - in consequence - the recruitment and activation of inflammatory proteins [11, 14, 46].

The urine of patients with GLN shows elevated levels of such cytokines, as IL-1, IL-6, IL-8, TNF-α and TGF-β. The transfer of mediators from the glomerulus to the tubule and interstitium triggers an induction of an immune response of the cell-mediated type [3, 14, 60, 72, 78]. Substances that exert such an effect include IL-1, which is said to induce ICAM-1 and VCAM expression in the tubular epithelium. Other mediators include TNF-β, IL-6 and LIF [78]. In consequence, the inflammatory process may develop and it is initiated by an immune reaction of the cell-mediated type [3, 14, 43]. Some role in its development may be played by the filtrate reaching the periglomerular and peritubular space [35].

Proteinuria

The most important damaging factor in chronic glomerulopathies is believed to be proteinuria. Interestingly, in numerous reports the degree of proteinuria is also the most potent clinical prognostic factor, while the associated interstitial changes are the most potent histological prognostic factor [6, 14, 52, 76]. Eddy [17] used a rat model and demonstrated a correlation between the intensity of interstitial infiltration, the degree of tubular damage and urine protein levels. The author did not explain the mechanisms underlying the phenomena he described; yet they have been described in other reports. A high protein content in the filtrate within the tubules is supposed to act through the following mechanisms [14, 26, 46, 53, 62, 72]:

- an increased reabsorption of protein from the tubular infiltrate that results in tubular cells overloading,

- an increased production of chemotactic mediators,
- an increased concentration of the elements of the complement in the urine, what allows for activating the system in the alternate pathway on the surface of the tubular brush border,
- an increased ammonia production resulting from an increased metabolism in the tubular cells, what also leads to complement activation,
- an increased transferrin secretion to the urine, what is associated with an increased iron ion concentration and generation of toxic hydroxyl radicals,
- an interaction between high levels of serum-originating and Tamm-Horsfall proteins, leading to blocking the tubules,
- components of the glomerular basement membrane penetrating the ultrafiltrate when podocytes are damaged.

Peruzzi et al. [58] demonstrated that a culture of epithelial cells of proximal tubules exposed to a high albumin concentration resulted in integrin βvα5 appearing on their surface. The authors observed this phenomenon both in cell cultures and in biopsy material in cases of glomerulopathy accompanied by proteinuria. Under normal conditions, tubular cells show only the presence of β3α1 and βvα3 integrins. The change of the phenotype is supposed to induce a sequence of further pathological phenomena in the interstitium. The role of integrins, and especially of β5α1, in the progression of glomerulopathy was also confirmed by the results obtained by Danilewicz et al. [15]. Tubular damage in glomerulonephritis may be detected morphologically as an increase in the number of mitoses and a transient vimentin expression [64]. It should be mentioned here that vimentin expression is also observed in the fetal development, so its appearance constitutes a return to a phenotype of a lower differentiation level. Grone et al. [24] found this intermediate filament in the epithelial cells of the renal tubule in both acute, experimental kidney damage and in humans with chronic renal diseases. They believed the change of the phenotype of a tubular epithelial cell to be merely a consequence of, and not an element involved in the pathogenesis of renal sclerosis. Nakatsuji et al. demonstrated that vimentin-positive and at the same time PCNA-positive (proliferating) tubular epithelial cells produced PDGF and osteopontin, with myofibroblasts appearing in the interstitium [16, 44].

Tubular epithelium

Tubular epithelial cells are supposed to demonstrate the activity of antigen-presenting cells, both with respect to foreign substances and autoantigens. These cells show a constitutive slight MHC-II expression. The low level of expression is increased by such mediators as INF-α. An increase in MHC-II expression may lead to the activation of immune responses of the cell-mediated reaction type. On the surface of tubular epithelial cells, numerous adhesion molecules can be found, such as ICAM-1 and VCAM-1. These cells produce some biologically active substances, as cytokines, T-lymphocyte stimulating factors, chemotactic and leukocyte-activating substances (PDGF, TGF-β, IL-1, IL-2, IL-6, IL-8, IL-15, MCP-1, TNF-α, RANTES, LIF, G-CSF, M-CSF, MCP-1 and osteopontin) [23, 62, 73].

Isbel et al. demonstrated and thoroughly documented the fact that the main source of M-CSF in experimental glomerulonephritis is the very tubular epithelial cell [29]. Roy-Chaudhury et al. [67, 68] observed in GLN the expression of LFA-1, VLA-4, L-selectin, ICAM-1 and VCAM-1 occurring within the tubular epithelium and interstitium, which was associated with interstitial inflammatory infiltration. The reason behind ICAM-1 expression may be the activity of INF-α. The appearance of adhesion molecules within the tubular epithelium indicated an association with the stage of chronic histological changes. On the other hand, Eddy et al. did not demonstrate an increased mRNA expression for MCP-1, ICAM-1 and VCAM-1 in the tubular epithelial cells in their model of proteinuria-induced nephropathy; according to the authors, the elevated quantity of these substances was to be predominantly dependent on their presence in inflammatory infiltrate cells [18].

Interstitial infiltration

An easily noticeable component of extraglomerular changes in glomerular kidney diseases is interstitial infiltration. A small number of macrophages may be seen in the renal interstitium under normal conditions, while in pathological states their amount is much greater [37]. Interstitial infiltration is believed to play a significant role in extraglomerular changes. In the majority of changes such infiltration is composed of macrophages and lymphoid cells, predominantly T lymphocytes. These cells demonstrate such activation-associated properties, as MHC-II or ICAM-1 expression [14]. The commonly accepted theory on the origin of macrophages in the interstitium holds it that the cells appear within the kidney with inflammatory lesions chiefly through their recruitment from the circulating blood. Nevertheless, it has been proved that a significant source of macrophages in numerous cases of inflammatory processes is their local proliferation. Such a situation may also take place in the kidney, as it was demonstrated by Lan et al. [36] in rats with glomerulopathy induced by antibodies to basement membranes of the glomerular capillary vessels. The authors found that the main source of macrophages in their model was proliferation. Most likely, M-CSF is the chief stimulator of macrophage proliferation; possibly also INF-γ is involved [72]. Akikusa et al. [2] studied interstitial infiltration and

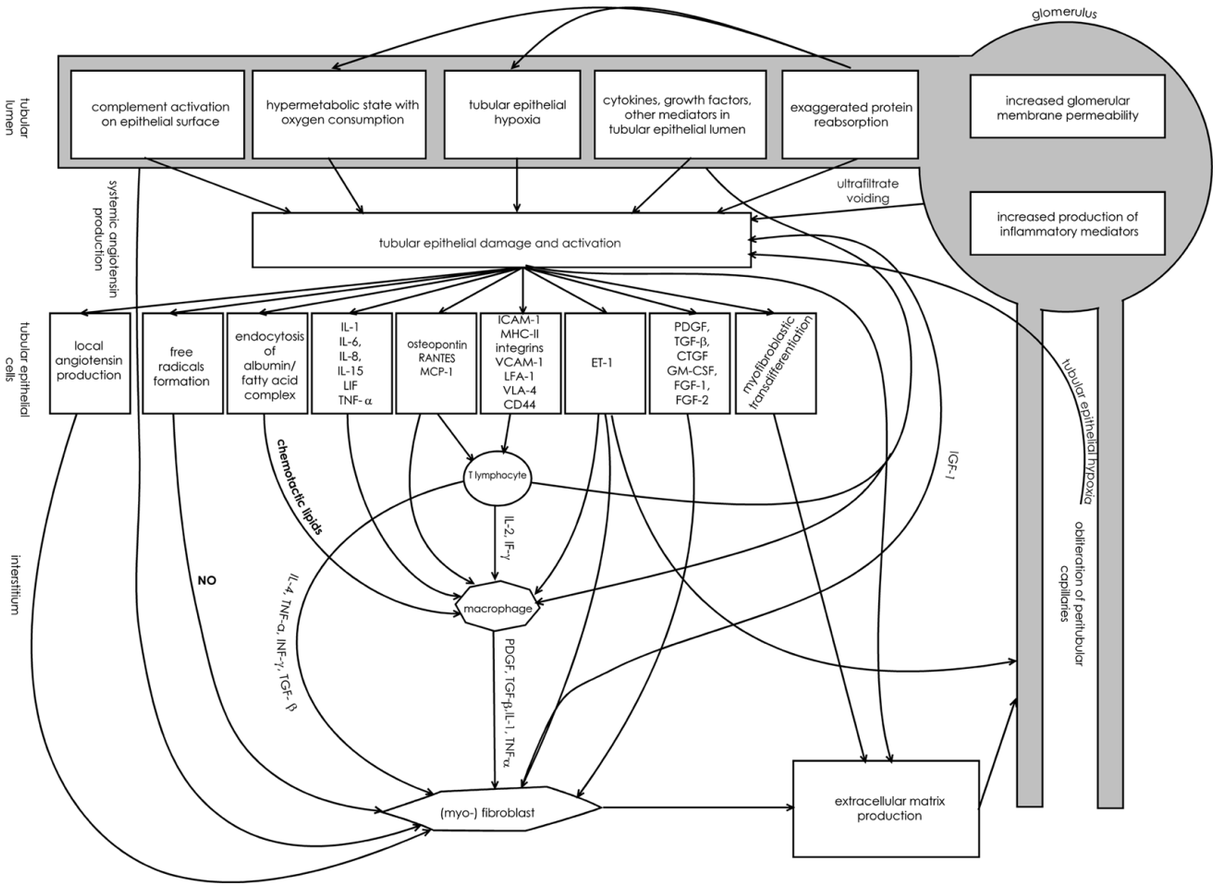

Fig. 1. Chief mediators of interstitial fibrosis in glomerulonephritis.

tubulitis in biopsy from patients with multi-organ vasculitis. The investigators observed that the intensity of inflammatory infiltration was to a certain degree associated with the grade of glomerular changes. Extraglomerular infiltration could not, nevertheless, be wholly explained by a direct spread of inflammatory infiltration originating in the glomeruli, as it was also noted in cases with no glomerular changes. The authors also showed a close correlation between tubulo-interstitial changes and the results of lab tests that evaluated tubular damage. Based on experimental models we might surmise that the appearance of infiltration composed of macrophages in the renal interstitium depends on the activity of T lymphocytes, both of the αβ and γδ type [56]. Important factors that are responsible for cellular infiltration are chemokines. Chemokine receptor antagonists reduce inflammatory infiltration and collagen deposition, possibly in part through their effect on TGF-β secretion. An increased expression of some CC chemokines and their receptors was demonstrated in a rat model of glomerulonephritis. Epithelial cells of the renal tubules are suggested to play a role in chemokine production, which is induced by protein, complement, lipoproteins and abnormal metabolites, such as free oxygen radicals, being secreted to the urine. According to Furuichi et al., the activity of chemokines was directly responsible for the development of interstitial changes in the course of glomerulopathy. Similarly, an increased expression of MCP-1 was noted in patients with membranous glomerulopathy. In a more advanced stage of the disease, chemokines are believed to originate in the inflammatory infiltrate cells themselves [22, 56, 70].

Myofibroblasts

An increase in the amount of extracellular matrix may occur both through an increase in the production of its components and through a decreased catabolism. A diminished resorption of extracellular matrix is said to result from the activity of tissue metalloproteinase inhibitor 1 or plasminogen activator inhibitor [18, 89]. Myofibroblasts are believed to be the chief sources of extracellular matrix in the renal interstitium. In glomerular diseases they become activated, what is manifested as desmin expression, among other phenomena [5]. The origin of myofibroblasts in the renal interstitium is not fully explained. Traditionally, the follow-

ing mechanisms are proposed: 1) a change in the interstitium fibroblast phenotype, 2) local proliferation, 3) the recruitment of myofibroblasts from the region surrounding blood vessels [37].

Theories for an alternate source of myofibroblast origin have also been proposed. In their report [48] Ng et al. pointed to a change in the direction of differentiation of tubular epithelial cells, mostly in the proximal tubule. Employing a model of subtotal nephrectomy in rats, the authors demonstrated the appearance of α-actin expression in the tubular epithelial cells, as well as structural changes that rendered epithelial cells similar to mesenchymal ones. A change in the phenotype of tubular epithelial cells was associated both with the number of interstitial myofibroblasts and with impaired renal function. Interestingly, these changes were the most pronounced at the sites where the basement membrane of a tubule was damaged. A mediator responsible for the transformation of tubular epithelial cells into myofibroblasts is believed to be TGF-β [20, 32].

An argument on behalf of an epithelial origin of renal interstitial myofibroblasts may be found in the publication of Nakatsui et al., who demonstrated that in the end-stage renal failure isolated cells with epithelial markers were found in the interstitium. The author explained the presence of these cells either by residual fragments of the tubules or by secondary epithelial differentiation within the interstitium. He offered no opinion on the possible co-expression of muscle (myofibroblastic) markers by isolated epithelial cells. A traditional explanation offered for the spatial proximity of myofibroblasts and activated tubular epithelial cells is the fact that the latter produce pro-myofibroblastic mediators [44].

The stimulation of interstitial fibroblasts may be induced both by inflammatory infiltrate cells (through TGF-β, IL-4), and by the activated tubular epithelium (through TGF-β, GM-CSF), or else *via* autocrine stimulation [23]. Apart from the increased proliferation and production of extracellular substance, some role in kidney scarring is also played by collagen production modulation: an increased amount of type III and I collagens with a simultaneous reduction in the production of type IV collagen. This is supposed to result in a changed structure of the basement membranes of the tubules, what is in turn accompanied by epithelial atrophy. The atrophy and fibrosis, once they start, become self-perpetuating, also in consequence of the autocrine fibroblast activity [23]. An additional, non-immune factor resulting in a further progression of kidney scarring is interstitial fibrosis that closes extraglomerular capillary vessels and causes ischemia [14].

Chief mediators

In numerous pathological processes the key mediator of fibrosis is TGF-β; other mediators include PDGF, bFGF, TNF-α and IL-1. Within the glomerulus, important mediators of cellular proliferation are PDGF, bFGF, IGF-1 and -2, produced by blood platelets, as well as PDGF, TGF-β, IL-1, TNF-α, IL-6 and bFGF, produced by macrophages. The most significant of them is PDGF. The presence of PDGF receptors was also demonstrated in fibroblasts of the renal interstitium [73]. Ostendorf et al. showed that inhibition of PDGF activity by specific antagonists arrested the development of antithymocyte serum-induced glomerulonephritis. Moreover, the authors found no increased TGF-β expression in the renal interstitium and no tubulo-interstitial changes. This suggests a primary role of PDGF in the development of scarring of the renal interstitium in this model [55]. In various glomerular diseases the role of particular growth factors and cytokines may be diversified. Thus, for example, although PDGF plays a significant role in the Heymann type glomerulonephritis, in membranous glomerulonephritis in humans only TGF-β, is implicated. On the other hand, PDGF is believed to be an important factor in the pathogenesis of glomerulopathies with a significant proliferative component, such as membranoproliferative or mesangial proliferative glomerulonephritis or else Berger disease [12, 55]. In TGF-β-transgenic rats the production of interstitial components by mesangial cells was markedly intensified, while their proliferation was moderate; at an increased PDGF expression, mesangial cells proliferate and the interstitium volume increases.

Another argument supporting the role of TGF-β can be found in the report of Border et al., who demonstrated that the inhibition of TGF-β activity by decorin resulted in a significant reduction in the degree of fibrosis in the antithymocyte glomerulonephritis model [9]. An increase of mRNA for TGF-β was also confirmed in the model of proteinuria-induced nephropathy [18]. In the activity of TGF-β, an increased expression of its receptors was also suggested to be a cofactor. Interestingly, TGF-β receptor expression in minimal change nephropathy is identical as in a healthy kidney. A mediator of TGF-β activity is connective tissue growth factor (CTGF). A distinct increase in CTGF expression was demonstrated in chronic nephropathies with a significant inflammatory component. The increase was correlated with renal scarring and cellular proliferation both in the interstitium and within the glomerulus. CTGF production is supposed to occur chiefly in the interstitial myofibroblasts and be TGF-β-dependent. According to Ito et al., CTGF is responsible for cellular proliferation and deposition of such substances as collagen I, fibronectin and β5 integrin [21, 30]. Wu et al. [77] demonstrated an increased TNF-β expression in membranous glomerulonephritis as compared to other types of glomerulonephritis. The expression of IL-1α, IL-2, IL-4, IL-8 and IL-10 was similar in both groups. Incidentally, IL-10 modulated and downregulated the Th1 lymphocyte-dependent response in the model of anti-GBM

glomerulonephritis in rats [34]. In some cases of minimal change glomerulopathy, peripheral lymphocytes become activated, what is manifested as an increased IL-4 expression; this phenomenon is not found in membranous glomerulopathy. Also in mesangial proliferative glomerulonephritis in humans there occurs an increase in TGF-β expression [79].

The signalling between the tubular epithelial cells and interstitial (myo)fibroblasts is reciprocal in nature; the epithelial cells act through TGF-β and PDGF and initiate the activity and proliferation of fibroblasts, while the latter act *via* IGF-1 and activate the epithelial cells [47]. On the other hand, the role of IGF-1 is not unequivocal, as in the model of reflux nephropathy it is believed to inhibit the activation of tubular epithelial cells and interstitial (myo)fibroblasts, as well as deposition of collagen [10].

As it follows from experimental data, the hepatocyte growth factor (HGF) participates in evoking a proliferative response of tubule cells to a damage [31]. The role of HGF was demonstrated *in vivo* in partial nephrectomy models and in models illustrating regeneration following acute tubular necrosis. On the other hand, HGF produced by tubular epithelial cells is supposed to affect the production of interstitial intercellular matrix, contrary to TGF-β [42].

Schaefer et al. [71] demonstrated an increased expression of connective tissue proteoglycans - decorin and biglycan - and their receptors in the glomeruli, as well as - what is more important - in the renal interstitium in a model of acute and chronic nephritis in rats. These compounds are believed to contribute to TGF-β-mediated renal fibrosis.

Osteopontin is believed to be an important mediator in the development of tubulo-interstitial changes in glomerulonephritis [18, 45, 52, 62, 72]. This glycoprotein is continuously produced by tubular epithelial cells and its production may be intensified by the administration of angiotensin II and through the activity of TNF-α, IL-1, TGF-β and vitamin D3 1,25$(OH)_2$. Osteopontin is a chemotactic agent and is macrophage-adhesive. An increased osteopontin expression was manifested in such disease models, as crescentic glomerulonephritis and systemic lupus erythematosus [54]. Osteopontin levels are correlated with the intensity of inflammatory process, including the intensity of histiocyte infiltration. Foci of interstitial infiltration are situated at sites of increased osteopontin expression in the tubular epithelium. In addition, the appearance of morphologically detectable tubulo-interstitial changes is preceded by the occurrence of osteopontin expression. Thus, osteopontin may act as an intermediary for cytokines, growth and hormonal factors that are produced in the glomeruli in the course of glomerulonephritis on the one hand, and interstitial changes on the other [11]. According to Panzer et al., in experimental glomerulonephritis osteopontin is the principal chemotactic factor in the interstitium [57]. Similar phenomena were also described in glomerular diseases in man [49, 52].

CD44 is a receptor for both hyaluronic acid and osteopontin. CD44 is found on the surface of the cellular membrane of proximal and distal tubular epithelium in a normal kidney; in pathological states its expression is intensified. Takazoe et al. demonstrated a variant of CD44, i.e. CD44V6, in a rat kidney. CD44V6 is characterized by an affinity not only to hyaluronic acid and osteopontin, buy also to chondroitin and heparan sulphate and may, therefore, differ in function from its standard form. CD44V6 expression in the tubular epithelium of the renal cortex is increased in pathological states, such as models of glomerulonephritis induced by antibodies to basement membranes or by urinary tract obstruction. Such an increased expression is especially visible at the sites of tubulo-interstitial damage. CD44 expression - both in its standard and V6 form - is IL-1 and TGF-β-dependent. The role of CD44 and CD44V6 in the pathogenesis of kidney diseases in humans has not yet been demostrated [74].

In experimental GLN an increased expression of SPARC is also observed in the interstitial fibroblasts. Such an increase precedes, and to a great degree is correlated with collagen I production, and thus with interstitial fibrosis. SPARC is especially strongly expressed in the vicinity of the tubules that show signs of atrophy [61].

Angiotensin

Angiotensin II and III is a very important mediator in the pathogenesis of glomerular kidney diseases. The importance of the renin-angiotensin-aldosterone axis in the regulation of arterial blood pressure is commonly recognized. In recent years, angiotensin has been demonstrated to be involved in the pathogenesis of primary kidney diseases through its effect exerted on the mesangial cells and fibroblasts of the interstitium. Within the glomerulus, angiotensin decreases filtration selectivity [62]. As it has been already mentioned, a low selectivity of proteinuria intensifies changes in the interstitium. In the kidney, angiotensin was found to induce c-fos, a protooncogen with a proliferative action. In addition expression of TGF-β increases production of fibronectin and other components of extracellular matrix [8, 59, 69]. The proliferative response differs in various elements of the kidney. Proliferation or an increased volume is described in the vascular smooth muscle cells and in mesangial cells. In interstitial fibroblasts proliferation occurs, while hypertrophy is seen in the proximal tubular epithelium [69]. Angiotensin also causes interstitial changes manifested as macrophage infiltration and fibrosis, while in the tubules the epithelium atrophies and the lumen is widened. Under the influence of angiotensin interstitial fibroblasts change their phenotype and are transformed into myofibroblasts [21, 41, 69].

Angiotensin may be produced and act not only in a systemic way; recently, the role of local angiotensin production has been emphasized. The expression of mRNA for angiotensinogen is observed in the tubular epithelial cells and interstitial fibroblasts, as well as in the mesangial cells. mRNA expression for angiotensinogen is intensified by angiotensin II and III through a feedback mechanism. Such local angiotensin generation leads to a diminished clinical effectiveness of angiotensin inhibitors (iACE) as compared to renoprotection, expected from *in vitro* studies [41, 69]. In addition to the "classic" angiotensin II, another factor that is involved in the pathogenesis of renal changes is angiotensin III, which has been traditionally believed to be a degradation product only. In fact, angiotensin II accounts only for 5 - 15% of the entire angiotensin activity in the kidney, what results from the high peptidase activity - predominantly of aminopeptidase A - in this organ. In pathological states, the activity of peptidases increases in consequence of the inflow of exogenous and local inflammatory cells. In addition, in various animal models of glomerulonephritis, the level of mRNA for aminopeptidase A was increased [69]. On the other hand, renin levels in glomerulonephritis were lower in comparison to the controls [63]. The above mentioned factors may lead to a relatively increased role of angiotensin III in primary kidney diseases.

In glomerulonephritis angiotensin convertase inhibitors (iACE) exert a confirmed positive effect on the prognosis. Some part of this effect is associated with normalized blood pressure, but at least in part the said effect is independent of blood pressure values. This is said to be related to the effect of angiotensin on the TGF-β synthesis, as well as on the kinin system. In addition, iACE reduce proteinuria both in man and in experimental models - possibly through acting directly on the filtration barrier. Other hypotensive drugs did not show such a significant renoprotective effect [59, 63, 75].

In IgA nephropathy angiotensin convertase gene polymorphism was demonstrated to affect the course of the disease. In D-allele patients, the disease is manifested earlier and progresses to chronic renal failure significantly more rapidly than in other affected individuals. The differences in the clinical course are clearly visible despite the lack of any differences in blood pressure values between the two groups [41]. A similar effect of ACE polymorphism on the clinical course was also observed in other glomerulopathies, such as focal and segmental sclerosis [21]. Van Essen et al. [19] also demonstrated a more rapid progression of renal failure in patients with DD ACE phenotype in several other chronic nephropathies. In contrast to individuals with II and ID phenotypes, these patients failed to derive any renal function benefit from iACE administration.

The pathogenesis of tubulo-interstitial lesions in GLN may be summarized as follows:

1. A damaging factor activates the tubular epithelial cells.
2. Subsequently, the interstitial cells are activated and they acquire the myofibroblast phenotype.
3. The myofibroblasts produce components of the extracellular matrix, what is manifested by a histologically detectable and measurable increased interstitial volume. The main mediators in these processes are TGF-β and PDGF.

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Adress for correspondence and reprint requests to:
K. Okoń M.D.
Department of Pathomorphology CMUJ
Grzegórzecka 16, 31-531 Kraków