Review

State-of-the-art strategies for the prevention of progression of glomerular disease to renal insufficiency

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Abstract

In this review, we present recent developments in the pathology of progressive glomerulosclerosis. Insights are based on evidence provided predominantly by us. We also describe a novel approach for studying local blockades of the renin-angiotensin system (RAS) and the cyclooxygenase-dependent pathway, with the objective to ameliorate progressive glomerulosclerosis. This method involves an *in vivo* imaging system using confocal laser scan microscopy.

Introduction

The renal glomerulus, an arterial microvasculature system, has been studied extensively, not only because it is a representative unit of physiological ultrafiltration but also in order to understand the mechanisms behind glomerular diseases, particularly progressive glomerulosclerosis, which can lead to "renal death." The number of patients with chronic renal insufficiency who need hemodialysis or renal transplantation has been on the rise everywhere [1]. In 2003, of more than one million patients worldwide, 320,000 were in the United States of America and were receiving maintenance dialysis [2]. In Japan, the corresponding number of such patients was 275,000 patients. The annual costs for maintenance dialysis were calculated to be more than 1.2 billion yen in 2007 [3]. It has been suggested that glomerular hemodynamic changes or glomerular growth responses may trigger the development of glomerulosclerosis, irrespective of etiology [4,5]. However, the cellular and molecular mechanisms behind progressive glomerulosclerosis remain unclear. The ultimate aim for any nephrologist is to prevent the progression of glomerulosclerosis to renal insufficiency, and the regression of sclerotic lesions in progressive, chronic glomerular diseases, such as diabetic nephropathy and IgA nephritis [6].

Animal models for clarifying the pathophysiological mechanisms underlying the progression of glomerular disease to renal insufficiency

We often use the rat as an experimental model, due to its suitability for pathophysiological or pharmacological research. Certain experimental rat models mimic irreversible human glomerulosclerosis. Of these, the 5/6 ablation

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model is the most commonly used and the most reliable. This model has also yielded the most informative results. For example, the demonstration that glomerular hyperfiltration, hyperfusion, hypertension, and hypertrophy are associated with the progression of glomerulosclerosis [4,5] originates from this experimental model. Other experimental models of progressive glomerulonephritis, such as the accelerated form of anti-glomerular basement membrane nephritis, which is characterized by destructive or crescentic glomerular lesions, differs substantially from human diabetic nephropathy and IgA nephropathy. In progressive experimental models of glomerulonephritis, a gradually accumulating mesangial matrix is observed.

In a previous study, we reported that progressive glomerulosclerosis can be induced in rats by a 1-shot injection of anti-Thy-1.1 monoclonal antibody (antithymocyte serum [ATS]), followed by unilateral nephrectomy [7]. The antibody binds to a specific epitope involved in endothelial-mesangial cell contact [8,9]. Several advantages of this experimental model were highlighted in the analysis of progression factors that lead to irreversible glomerulosclerosis. As shown in Fig.1, first, the course of disease between nephrectomized (1-kidney) and sham-operated (2-kidney) groups of rats are comparable, at least at the theoretical level, since the same amount of nephritogenic antibody is bound to each kidney. Second, there is a sharp difference in the prognosis of disease between the 1-kidney and the 2-kidney model. The 1-kidney model is characterized by progressive glomerulosclerotic lesions with renal insufficiency, while the 2-kidney model is fundamentally reversible [10]. Only one procedure of unilateral nephrectomy after the disease induction resulted in sharply different prognosis. Third, the model can be applied to different rat strains, including Munich Wistar rats, in which many glomeruli are located directly under the vicinal surface of the kidney cortex [11,12], and transgenic Sprague Dawley rats carrying the enhanced green fluorescent protein (EGFP) gene [13,14].

Diabetic nephropathy is the most common causal disease leading to renal insufficiency. Patients need regular hemodialysis treatment or renal transplantation. Animal models with the physiological and pathological characteristics of diabetic nephropathy are important in further clarifying the current understanding of the pathogenic mechanisms of progression, and to

Figure 1. Scheme showing the disease courses of the 1-kidney and 2-kidney models (reference 5)
Anti-Thy-1 nephritis was induced in rats by intravenous injection of 0.5 mg of the anti-Thy-1.1 monoclonal antibody 1-22-3. Thirty minutes after injection, unilateral nephrectomy of the right kidney was performed (1-kidney model). In the 2-kidney model, a sham operation was performed 30 minutes after injection of the same dose of antibody.

examine the effect of potential renoprotective agents. Rat models with chemically induced diabetes such as streptozotocin (STZ)-induced diabetes and genetic diabetes such as the Zucker diabetic fatty rat are widely used for this purpose [15,16].

Risk factors involved in the progression to glomerulosclerosis

The human circulatory system maintains homeostasis inside the body by distributing blood in a dose-dependent manner, according to oxygen and nutrient requirement. Preserving vascular function requires a good balance in whole-body physiology. This is certainly also true for the renal glomerulus, as a representative unit of arterial microvasculature. Glomeruli are composed of endothelial cells, mesangial cells, and mesangial matrices. Upon glomerular damage, glomerular function may be impaired before effective repair of the glomerular cells and matrix components occurs We showed that impairment of vascular regeneration is strongly associated with the development of progressive glomerulosclerosis in the 1-kidney model, compared to the 2-kidney model (when induced by a 1-shot injection of ATS) [7]. Semi-quantitative analysis revealed that the capillary density and mRNA expression of platelet/endothelial cell adhesion molecule (PECAM)-1, vascular cell adhesion molecule (VCAM)-1, and vascular endothelial growth factor (VEGF) in the glomeruli, are significantly reduced in the 1-kidney group on day 14 (an early stage of glomerulonephritis), compared to the 2-kidney group. On day 84, the 1-kidney group exhibited progressive glomerulosclerotic lesions, followed by a decrease in capillary density. In contrast, the glomerular architecture of the 2-kidney group recovered to an almost normal structure.

Using EGFP-positive bone marrow (BM) chimeric rats in the same way as described above, progressive glomerulosclerosis was induced by a 1-shot injection of ATS, followed by unilateral nephrectomy (1-kidney model) [14]. We examined the recruitment of BM-derived cells in isolated glomeruli and frozen sections. A confocal laser scan microscopic study revealed that BM-derived PECAM-1^+RECA-1^+ endothelial cells and OX-7^+ mesangial cells contribute to structural support for the glomerular capillaries during the chronic stage of the disease [14].

There is increasing evidence that changes in glomerular hemodynamics may promote the development of glomerulosclerosis, irrespective of etiology [4,5]. Direct and real-time observation of the hemodynamic events occurring in the microcirculation such as the glomerular capillary tuft, were extremely challenging due to limitations of the available microscope optics. One observation of the glomerular microvasculature in the rat was performed using hydronephrotic kidneys [17]. However, hydronephrosis is accompanied by a marked decrease in kidney blood flow [18-20]. To avoid non-physiological effects of operative procedure, we introduced an intravital real-time confocal laser-scanning microscope (CLSM) system, in combination with a high-speed video camera and fluorescent tracer labeling [21]. At first, we confirmed that the destruction of the mesangial supporting system of the glomerular vasculature, induced by an intravenous injection of ATS, resulted in abnormal hemodynamics within the limited tufts of glomeruli [21]. Next, the sequence of hemodynamic changes within the glomeruli was analyzed in the reversible 2-kidney and irreversible 1-kidney ATS models [22]. The determining point in glomerulosclerosis progression occurs between day 7 and day 14 after disease induction, when disturbances of local intraglomerular blood flow persist in the 1-kidney group. It should be pointed out that disturbance of local intraglomerular blood flow, which we called “turbulence of glomerular hemodynamics,” significantly precedes

progressive glomerulosclerosis, observed 84 days after disease induction. From the viewpoint of hemodynamics, it is well known that turbulent flow requires more pressure for a given flow rate than laminar flow does [23]. Therefore, it is reasonable to consider that disturbances in intraglomerular blood flow in the 1-kidney model may induce higher shear and hydrostatic stress along the glomerular capillary walls in the early phase of renal disease, leading to retardation of capillary repair and finally, to progressive glomerulosclerosis.

Therapeutic strategies to halt progressive glomerulosclerosis: a search focusing on local delivery of drugs

In clinical situations, drug therapies for glomerulonephritis should be provided after the nephritic symptoms are diagnosed. Therefore, it is important to identify the point that determines whether the progression of glomerulosclerosis is reversible or irreversible. As described in the above section, the turning point from progressive glomerulonephritis to irreversible glomerulosclerosis was determined to be 7 to 14 days after disease induction, at a fully established stage of glomerular inflammation.

As shown in Fig.2, we focused on developing therapeutic approaches that target 3 possible mechanisms involved in the progression of glomerulosclerosis. First, we targeted BM-derived stem cells to stimulate recovery from

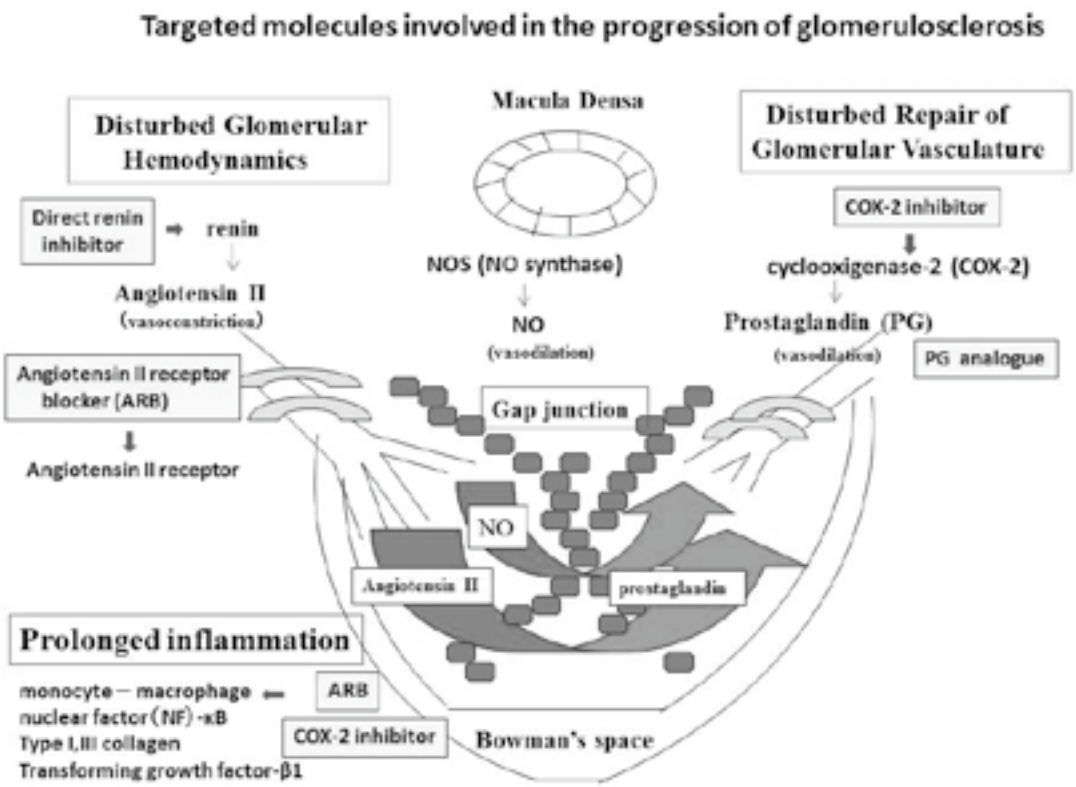

Figure 2. Mechanisms involved in the progression of glomerulosclerosis
Schema of juxtaglomerular apparatus and glomerulus is shown in addition to the key molecules regulating the glomerular hemodynamics. AII; angiotensin II, COX-2; cyclooxygenase-2, NO; nitric oxide, PG; prostaglandin, ARB; angiotensin II blocker.

progressive glomerulosclerosis [24]. $EGFP^-$ BM chimeric rats were infused with BM cells from $EGFP^+$ donors on day 7 after induction of the 1-kidney model of ATS glomerulonephritis. BM cell infusion improved renal function and glomerular hemodynamics and ameliorated histological alterations with reduced glomerular infiltration of macrophages, leading to a dramatic reduction in mortality: only 2 of 8 untreated rats survived 12 weeks after disease induction, while 5 of 6 BM cell-infused rats survived till the 12th week. In the BM cell-infused group, urinary protein excretion, serum creatinine, and blood urea nitrogen levels were significantly lower than in the untreated group from day 8 until 12 weeks after disease induction.

RAS-blocking drugs are known to exert beneficial effects on the progression of glomerular diseases that would otherwise result in renal insufficiency [25]. There is also increasing evidence that intrarenal RAS within glomeruli and interstitial tissues, including renal tubules, is not only involved in physiological processes but also in pathological conditions, such as vascular remodeling, diabetic nephropathy, and glomerulosclerosis [26-29]. Therefore, we attempted to block intrarenal RAS by using a novel approach that applies renal subcapsular implantation of a collagen sponge as a means to deliver angiotensin II receptor blocker (ARB) or direct renin inhibitor (DRI) locally [30,31]. At day 7 after induction of the 1-kidney model of ATS glomerulonephritis, pellets of type-1 collagen containing phosphate-buffered saline, ARB (Valsartan), angiotensin II, or direct renin inhibitor (Aliskiren), were implanted into the subrenal capsular pocket. Local ARB or DRI treatment significantly reduced proteinuria and ameliorated glomerular pathology, including glomerular matrix expansion and the sclerotic index. In addition, glomerular blood flow levels were also significantly improved compared to those in the untreated disease group [30,31].

Using STZ-induced diabetic rats, we examined the effects of a selective ARB on renal hemodynamic changes [32]. The diameters of afferent and efferent arterioles, erythrocyte velocities within glomeruli, and volume flow in the glomerular capillary loops in 4-day diabetes mellitus (DM), were significantly higher than in control rats, and these increases were even more pronounced in 28-day DM. The ratio of the diameter of the afferent artery to the diameter of the efferent artery in 28-day DM, 1.11, was markedly increased when compared to control and 4-day DM groups, 1.05 and 1.07, respectively, indicating a continuous increase in internal glomerular pressure with time. Local ARB treatment improved all these measurements and significantly decreased proteinuria, while the blood glucose level remained unchanged.

Prostaglandins (PGs) modulate renal function, including hemodynamics and water and salt homeostasis. These eicosanoids are formed by cyclooxygenase (COX)-dependent metabolism of arachidonic acid. COX-2 is constitutively expressed in the macula densa of the juxtaglomerular apparatus and in adjacent epithelial cells of the cortical thick ascending limb of loop of Henle [33]. We examined the pharmacological effects of 2 contrast agents (a selective COX-2 inhibitor and a prostacyclin analogue, PGI_2) on the course of the disease in the 1-kidney model of ATS glomerulonephritis [34]. The local delivery of a COX-2 inhibitor at day 7 after disease induction slowed the progression of glomerulosclerosis. However, local administration of PGI_2 induces vasodilation and consequent glomerular hyperperfusion. PGI_2 is, therefore, considered a factor inducing a vicious circle in progressive glomerulosclerosis. These results are consistent with our working hypothesis that disturbance of intraglomerular microcirculation is a risk factor for progressive glomerulosclerosis [35].

Next, we examined the renoprotective effects

of drugs with regard to the inflammatory aspects. Local delivery of ARB in STZ-induced diabetic rats attenuated monocyte/macrophage infiltration in the glomeruli and reduced the glomerular expression and translation of endothelial nitric oxide (NO) synthesis genes [36]. Activation of NF-κB, a well-known player in inflammation and in the immune response, can be measured as the increase in expression of the p65 subunit of NF-κB. Local ARB treatment induced an apparent reduction in nuclear-localized p65 and in the intensity of the staining. Furthermore, glomerular expression of the breakdown products of 145/150-kDa spectrin, a specific marker of calpain activation, was dramatically increased in diabetic rats, while the protein expression reverted to normal levels after local ARB treatment. These results indicated that ARB acts as an anti-inflammatory drug during the early phases of STZ-induced diabetic nephropathy.

The pharmacological effects of local delivery of DRI or COX-2 inhibitor in the 1-kidney model of ATS were evaluated. Semi-quantitative and quantitative analysis of immunohistological findings showed that both treatments significantly suppressed mesangial matrix expansion and improved glomerular sclerotic index compared to the diseased control, representing pathological expressions of α-SMA and type 1 collagen [31,34].

Concluding remarks

In this article, we describe state-of-the-art strategies for preventing the progression of glomerular diseases to renal insufficiency. Local delivery of renoprotective agents such as RAS and COX-2 inhibitors can be applied to successfully control glomerular hemodynamics and to attenuate glomerular inflammation. Furthermore, we introduced a new imaging system, which enables us to observe glomerular circulation. As it is widely accepted that “Seeing is believing”, this noninvasive diagnostic device can contribute to the analysis of glomerular function parameters, including hemodynamics and permeability, enabling us to objectively diagnose the stage of progression, and to examine the pharmacological effect of established or newly developed drugs. Further work and time will be needed to extrapolate our knowledge from in vitro, ex vivo, and in vivo experiments to a full clarification of pathogenesis and therapy in humans.

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