Genetics and pathogenesis of progressive glomerulosclerosis

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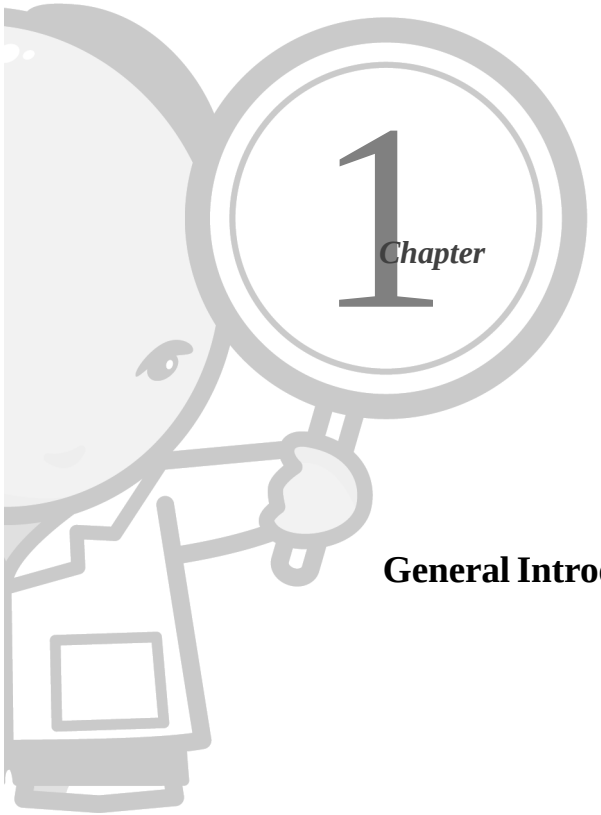

Chapter 1

General Introduction

General introduction

Glomerulosclerosis is a general term describing the process of scarring of the glomeruli, the functional units in the kidney that filter urine from the blood. This severe, irreversible complication can occur secondary to various already established systemic or local diseases. However, not all patients with renal diseases show progression to end stage renal disease (ESRD). Thus renal patients can be subdivided into progressors and non-progressors based on clinical parameters[1]. Why patients with renal diseases become progressors or non-progressors is unclear, and better insight into the pathogenesis of glomerulosclerosis may improve our understanding of the process towards progression. However, the pathogenesis of glomerulosclerosis is complex and still poorly understood, although genetic factors probably play a role, given the considerable variation among individuals in both the risk of developing glomerulosclerosis and the rate of progression. Therefore, the first aim of the work described in this thesis was to identify genes involved in the progression and repair of glomerulosclerosis, using an animal model that allows a clear distinction between progression and repair after renal injury. The second aim was to gain better insight into the pathogenesis of glomerulosclerosis by investigating the expression and activity of fibrosis-related molecules in an animal model and in patients with renal diseases.

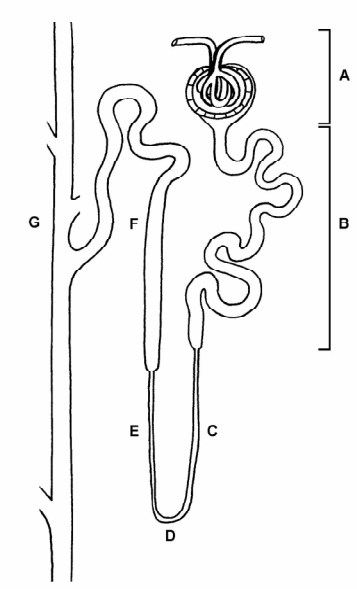

Figure 1. Schematic drawing of the nephron. Glomerulus (A), proximal tubule (B), descending thin limb (C), Henle's loop (D), ascending thin limb (E), distal tubule (F), and collecting duct (G).

Anatomy and functions of the kidney

The kidneys are excretory organs that work in collaboration with the lungs, the skin, and the liver in maintaining the body's homeostasis. Via blood filtration, the kidneys clear the body of many organic wastes and of ammonia, urea, and other metabolic byproducts that would otherwise be harmful if not excreted. In humans, the kidneys are bean-shaped organs located in the retroperitoneum. A single kidney is about 12 cm long, 6 cm wide, and 3 cm thick, weighs approximately 150 grams, and contains about one million nephrons. The nephron is the basic structural and functional unit of the kidney, composed of an initial filtering component, called the glomerulus, and a tubule specialized in reabsorption and secretion (Fig. 1). The blood supply to the glomerulus arrives via an afferent arteriole, and blood pressure is the driving force behind the filtering process. Filtered blood passes into the efferent arteriole.

Glomerular blood flow is regulated by the juxtaglomerular apparatus, which consists of juxtaglomerular cells, the macula densa, and extraglomerular mesangial cells. Juxtaglomerular cells, also known as granular cells, are the sites of renin secretion. The juxtaglomerular cells are found in the walls of afferent arterioles of the glomerulus and act as an intra-renal pressure sensor. Decreased pressure leads to renin secretion, which acts to increase systemic blood pressure. The macula densa senses fluid flow rates as well as sodium chloride concentration in the distal tubule. Subsequently, the macula densa secretes a locally active vasopressor, which acts on the adjacent afferent arteriole to decrease glomerular blood flow. The extraglomerular mesangial cells, also known as lacis or Goormaghtigh cells, lie sandwiched between the macula densa and the afferent and efferent arterioles. The role of extraglomerular mesangial cells in controlling the glomerular blood flow is still poorly understood[2].

The glomerulus (Fig. 2A) is surrounded by the Bowman's capsule, which is lined by parietal epithelium. The visceral layer, made of podocytes, is attached to the glomerular basement membrane (GBM), which lies between the layer of visceral epithelial cells and fenestrated endothelial cells, forming the inner lining of the glomerular capillary tuft (Fig. 2B). The GBM is a negatively charged network consisting among other things of collagen, laminin, and heparan sulfate proteoglycans. Podocytes, as their name implies, cover the glomerular capillary walls by means of foot processes, and slit pore membranes bridge the slits between the foot processes at their GBM attachment. Negatively charged glycosylated macromolecules known as the glycocalyx cover the podocytes. The glycocalyx is thought to be important in the maintenance of foot process structures and in glomerular size and charge selectivity[3].

The mesangium is located between the glomerular capillaries. It consists of intraglomerular mesangial cells and mesangial extracellular matrix (ECM). Mesangial cells play a critical role in maintaining the structural integrity of the glomerular tuft and in modulating blood flow by relaxation or contraction[4]. Three layers form the filtration barrier: the GBM, the slit pore membranes, and the glycocalyx produced by podocytes. The size of the endothelial cell fenestrae restricts passage of large (beyond 70 kD) molecules and cells (e.g., red blood cells, leukocytes, and platelets). In addition, the negative charge of the GBM and foot processes limits filtration of negatively charged molecules (e.g., albumin). Overall, the size, charge, and configuration determine the passage of each molecule[5;6]. The resulting ultrafiltrate collects in the space between the glomerular tuft and Bowman's capsule called the Bowman's space, which opens into the proximal tubule. Subsequently, the ultrafiltrate runs through the thin descending limb, Henle's loop, the thin ascending limb, the thick ascending limb, the distal tubule, and eventually into the collecting tubules (Fig. 1). In contrast to distal tubular epithelial cells, proximal tubular epithelial cells are characterized by microvillous membranes, called brush border, at the luminal side of the tubule. Reabsorption mainly of water, glucose, and amino acids takes place in the renal tubules, resulting in the production of approximately 1 to 1.5 liters of urine each day[7-9].

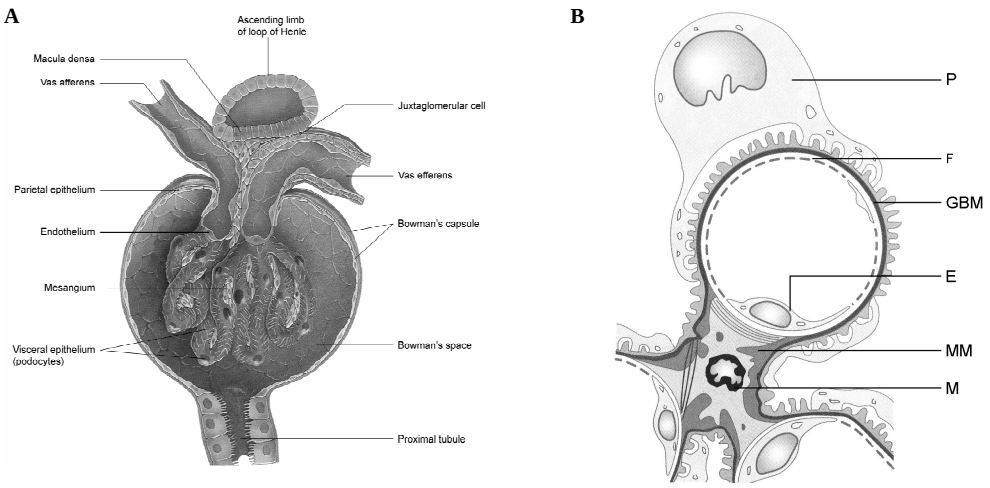

Figure 2. A. Schematic drawing of the glomerulus. Illustration modified and printed with permission from Tortora *et al.*, Principles of Anatomy and Physiology. In.: *Anatomy and Histology of the Kidney*, 11th Edition., USA, Wiley, 2006, pp. 992–1055. B. Schematic drawing of the mesangial region surrounded by capillaries. Mesangial cell (M), mesangial matrix (MM), endothelial cell (E), glomerular basement membrane (GBM), podocytes (P), and fenestration between endothelial cells (F). Illustration modified and printed with permission from van der Meer *et al.*: Interne geneeskunde. In: *Nierziekten*, 12th Edition, Houten, Bohn Stafleu Van Loghum, 2001, pp. 321–373.

Renal injury

The wide variety of different clinical symptoms that result in glomerular injury reflects the functional complexity of the glomerulus. Laboratory test results often provide the first and only indications of a renal insufficiency; however, a specific anamnesis and physical examination are crucial in determining the type of renal disease. The clinical signs of renal diseases are decreased glomerular filtration rate (GFR), micro- or macroscopic hematuria (erythrocytes in urine), proteinuria (increased protein concentration in urine), disturbed renal function (elevated plasma creatinine levels), and hypertension.

Renal Syndromes

Although there are numerous renal disease states and conditions that lead to altered kidney function, there are relatively few renal syndromes. Disease processes that alter kidney function must cause abnormalities in the glomeruli, tubules or interstitial areas (tubulointerstitial areas), or renal vasculature. The tubular and vascular syndromes will not be addressed in this thesis. Below is a list of the major glomerular syndromes[10-12].

- Nephritic syndrome, characterized by hematuria, variable degrees of proteinuria (< 3.5 g/day), oliguria, hypertension, and deterioration of renal function
- Nephrotic syndrome, clinically characterized by severe proteinuria (> 3.5 g/day), hypoalbuminemia, edema, hyperlipidemia, and hypercoagulability
- Isolated hematuria and asymptomatic proteinuria
- Rapidly progressive glomerulonephritis, the most severe form of nephritic syndrome
- Renal failure or renal insufficiency, described as a decrease in GFR that manifests clinically as elevated serum creatinine

Renal failure can broadly be divided into two categories: acute renal failure and chronic renal failure. Acute renal failure refers to an acute decline in renal function over a short period of time. In contrast, chronic renal failure develops slowly and with few symptoms initially. Chronic renal failure can result in a decreased number of functioning nephrons or a slow decrease in GFR, and tends to be progressive. ESRD is the ultimate outcome and is commonly characterized by progressive sclerosis of glomeruli and interstitium. This process is often irreversible, necessitating hemodialysis until a renal transplant donor is found[13].

Glomerular diseases

Glomerular diseases are characterized by primary abnormalities of the glomerulus, both (ultra)structural (cellular proliferation, inflammation, epithelial cell changes, basement membrane thickening, fibrosis, obliteration of epithelial cell pedicles) and functional (increased

permeability resulting in proteinuria or hemorrhage of glomerular origin), and can be acute or chronic. The classification of glomerular diseases is complicated and still evolving and uses a combination of clinical (congenital or acquired; acute or chronic), morphologic (proliferative, membranous, minimal changes), and immunologic criteria. Below is a list of the major glomerular diseases[14-19].

- Congenital glomerular diseases (e.g., hereditary nephritis, including Alport's syndrome, and congenital nephrotic syndrome)
- Primary acquired glomerular diseases, in which renal involvement is the main manifestation of the disease (e.g., minimal change disease, proliferative glomerulonephritis, membranous glomerulonephritis, membranoproliferative glomerulonephritis, focal glomerulonephritis)
- Secondary acquired glomerular diseases, in which renal involvement is part of a systemic disease such as systemic lupus erythematosus or progressive systemic sclerosis
- Other glomerular diseases (e.g., diabetic nephropathy, amyloidosis)

Most forms of primary glomerular diseases are the result of immune complex formations, most of which arise via two distinct mechanisms. The first mechanism is local binding of antibodies to renal antigens (e.g., epithelial, endothelial, and mesangial cell antigens, or ECM antigens), or local binding of antibodies to antigens planted on pre-existing glomerular structures. The second mechanism is deposition of circulating macromolecular immunoaggregates within the glomerulus. Immune complexes and antibodies that are deposited activate the classical complement cascade, in which C1q is involved. Subsequently, granulocytes and platelets are bound to the site of deposition, followed by the release of complement factors C3a and C5a, which recruit leucocytes (macrophages and monocytes). Degranulation of the granulocytes and platelets results in protease and oxygen radical release, causing lysis of cell membranes and matrix components. In addition to chemotactic properties, the complement system can be cytotoxic by itself. Assembly of the complement components C5b–9 on the target cell forms the cell membrane attack complex. As a consequence, the cell will be irreversibly damaged. The glomerular compartment (endothelial, epithelial, or mesangial) in which the inflammation process is active largely determines the type of glomerulonephritis.

Injury to the capillary endothelial cell can lead to an influx of leucocytes, thrombosis, thickening of the endothelial layer, necrosis, and intra- and extra capillary cell proliferation. Clinical characteristics are hematuria, proteinuria, and a decrease in GFR. This type of injury is seen in anti-GBM glomerulonephritis and lupus nephritis class III and class IV[20;21].

Epithelial cell injury can result from formation of subepithelial immune complexes. Clinically, patients with this type of injury develop a nephrotic syndrome, without or with mild hematuria and with normal GFR. Subepithelial immune complex depositions may lead to the formation of nail-like projections from the GBM called spikes. In addition, visceral epithelial cell damage occurs, without infiltration of leucocytes or cell proliferation. An example of this type of injury is membranous glomerulopathy, the most common cause of nephrotic syndrome in adults. Minimal change disease (MCD) is the most common cause of nephrotic syndrome in children and is another example of epithelial cell injury[14]. MCD is defined by severe proteinuria in conjunction with normal glomeruli, the absence of interstitial fibrosis, and obliteration of glomerular epithelial cell pedicles, as confirmed by electron microscopy.

Mesangial cell injury can result from immune complex binding to mesangial cell-specific receptors, the binding of mesangial cell-specific antibodies, or trapping of immune complexes in the mesangium[22]. As a result, the mesangial cells are activated and will start to proliferate, producing an increase in matrix production. This type of injury is clinically characterized by asymptomatic proteinuria and macroscopic or microscopic hematuria with a normal or slowly decreasing GFR. The most common type of mesangioproliferative glomerulonephritis is IgA nephropathy, also known as Berger's disease[23]. The disease can be progressive, resulting in ESRD necessitating kidney transplantation. IgA nephropathy may return in the transplanted kidney, but loss of renal function is rare.

Injury of non-immunological origin (e.g., hypertension, hyperglycemia, and hyperlipidemia) can also cause glomerular diseases[24-26]. The kidney can both contribute to and be a target of hypertension (renal hypertension and systemic hypertension, respectively). Systemic blood pressure elevation is associated with increased vascular tone resulting from decreased production of vasodilator molecules, such as endothelium-derived nitric oxide. At the same time, there is increased production of vasoconstrictors, such as angiotensin II and endothelin-1. Functional changes that result from hypertension include a decline in GFR and abnormalities in tubular function, including new onset or worsening of proteinuria. These functional changes result from structural changes in the GBM, expansion of the mesangial and interstitial matrix, ultimately resulting in sclerosis of both glomerular and tubular elements. When the compensatory capacity of the remaining nephrons is exceeded, renal function progressively deteriorates and renal failure develops[7;27;28]. Diabetic nephropathy, an example of glomerular injury caused by injury of non-immunological origin, is the main cause of end-stage renal failure in many countries and results from interplay between hemodynamic changes (hypertension) and metabolic changes (hyperlipidemia). Features of diabetic nephropathy are hypercellularity, thickened basement membrane, and increased

mesangial matrix. Glomerulosclerosis and vascular diseases are the main causes of renal failure in diabetic patients[29].

Glomerulosclerosis

Regardless of the underlying pathogenetic mechanisms of a renal disease, progression to ESRD follows a final common pathway culminating in glomerulosclerosis and tubulointerstitial fibrosis[30]. Glomerulosclerosis results primarily from a perpetuated activation of fibroblasts, overproduction of ECM, and reduced ECM degradation[28;31-34].

Examples of glomerular diseases with glomerulosclerosis are global glomerulosclerosis and focal and segmental glomerulosclerosis (FSGS). FSGS is a clinico-pathological disorder morphologically characterized by a segmental solidification (sclerosis) of the glomerular tuft resulting from focal collapse of the glomerular capillary wall. Clinical features include asymptomatic proteinuria or nephrotic syndrome with or without renal insufficiency, and microscopic hematuria is frequent[35]. To better comprehend some aspects of pathogenesis, FSGS can, based on etiological differences, be classified as follows:

- Primary (or idiopathic) FSGS, of an unknown etiology and subject to an ongoing debate about its status as a distinct entity
- Secondary FSGS, including the following subcategories: familial/genetic, virus-associated, drug-induced, and mediated by adaptive structural-functional responses

Over the last two decades, a growing number of publications have addressed the morphological heterogeneity of primary and secondary FSGS[36;37]. Recently, d'Agati *et al.* proposed an FSGS subclassification based on morphological features. The five morphological variants of FSGS include FSGS not otherwise specified (NOS), and the perihilar, cellular, tip, and collapsing variants. Classification of FSGS can now involve designation of a morphological category and, when possible, an etiologic category. However, how the different morphological subtypes of FSGS reflect differences in etiology, pathogenesis, prognosis, or optimal therapy remains to be determined[38].

Genetic factors in kidney diseases

In some cases the relationship between genetics and renal disease development is evident. Examples are familial forms of FSGS caused by mutations in the podocyte molecules podocin, CD2-associated protein, alpha-actinin-4, or the canonical transient receptor potential 6[39-42]. Screening for mutations in the abovementioned genes in sporadic cases of nephrotic syndrome has provided new insights and is increasingly being integrated into pediatric nephrology[43].

Genetic factors frequently have a less direct influence on renal disease development and become manifest only in the presence of "permissive conditions" such as diabetes mellitus and hypertension. Conversely, not all patients suffering from these conditions develop renal disease or progress to ESRD, and it is likely that genetic factors determine the time of onset and the rate of progression of kidney diseases. For example, several genetic linkage analyses in diabetic nephropathy have shown a susceptibility locus on chromosome 18q[44;45]. A polymorphism in the DNA sequence of the *CNDP1* gene, which encodes the enzyme carnosinase-1, on chromosome 18q in diabetic patients determines susceptibility to developing diabetic nephropathy[46]. The substrate of carnosinase-1, L-carnosine, is a potent inhibitor of oxidative stress[47] and the formation of advanced glycation end products[48], and it may thus act as a cytoprotective factor in diabetes mellitus. It has been postulated that opposing mechanisms, i.e., hyperglycemia *versus* the action of protective factors such as L-carnosine, determine the net outcome of diabetic nephropathy[46].

Natural genetic heterogeneity among individuals impedes the identification of genes marking a predisposition to progressive renal disease in humans. Investigation of animal models, through comparison of strains that are progressors with those that are not, may circumvent these problems. Identified candidate genes in animal models could eventually be of relevance in human populations. For example, researchers have identified two Lewis rat sub-strains with small genetic differences but with a considerable difference in susceptibility to developing progressive glomerulosclerosis after induction of a mesangial inflammatory disease called "anti-Thy-1 glomerulonephritis"[49]. The following section gives a detailed description of the anti-Thy-1 nephritis model.

Because chromosomal regions identified by linkage analyses generally contain tens to hundreds of genes, pinpointing the exact genes affected in any particular disease is an elaborate task. Mutations in the DNA sequence of such genes may give rise to altered gene expression levels. Therefore, an alternative approach to identifying genes involved in progression or remodeling of damage to renal tissue is the application of genome-wide gene expression analysis using microarrays. The microarray technique enables simultaneous monitoring of expression levels for thousands of genes. Chapter 4 provides an example of gene expression analysis using a microarray. Overall, identification of genes determining disease progression will become more rapid and specific with the combined application of genetic linkage analysis and gene expression profiling [50].

Anti-Thy-1 nephritis

Numerous experimental disease models have been established in rats and mice with the goal of mirroring various elements of human renal diseases. One particular example is the anti-Thy-1 nephritis model, commonly referred to as anti-Thy-1 glomerulonephritis. This rat model is frequently used to simulate mesangial injury, and the disease can be induced by injection of rats with antibodies against Thy1.1, a transmembrane protein expressed on mesangial cells. The Thy-1 antigen was one of the first lymphocyte-differentiation antigens to be discovered in the mouse[51]. In the mouse, Thy-1 is present on thymocytes, thymus-derived peripheral lymphocytes, and on bone marrow cells[52]. In contrast to the mouse, which is known to carry the Thy-1 antigen in two allelic forms, only one allele (Thy-1.1) has been found in the rat[53]. In rats, Thy-1.1 is found on thymocytes and a subset of nucleated bone marrow cells. Contrary to mouse, most peripheral T lymphocytes from rats are Thy-1 negative[54]. In rats, the Thy-1 antigen is also present on the glomerular mesangium, the medullary collecting tubular basement membranes, and the pericytic sheath of the vessels[55;56].

Anti-Thy-1 nephritis is induced by a single intravenous injection in rats of mouse monoclonal anti-rat Thy1.1 antibody, which results in an acute glomerulonephritis. The disease is characterized by direct binding of the antibody to the GBM and mesangium, followed by immediate activation of the complement system, as reflected by glomerular deposition of the complement factors C3 and C9 and the presence of the C5–C9 membrane attack complex. Furthermore, the coagulation cascade is activated, represented by the presence of fibrinogen deposition in the affected glomerulus. One hour after injection, mesangial alterations are prominent, including condensation of mesangial cell chromatin and lysis of mesangial cells. After 24 hours, an increased influx of inflammatory cells (macrophages and polymorphic nuclear cells) can be observed in the glomeruli. After 4 to 7 days, mesangial cell proliferation is prominent, accompanied by the formation of aneurysms in the capillary tuft and glomerular hypercellularity. Subsequently, the glomeruli show an increase of mesangial cells in the mesangial areas, and extracapillary proliferation leading to glomerular crescent formation at day 14, which decreases gradually 3 weeks after disease induction.

The disease is clinically characterized by a massive transient proteinuria starting immediately after antibody injection, reaching maximum values of ±300 mg/24 h between days 2 and 7, and gradually decreasing to normal levels after 3 weeks[57]. Overall, renal function and structure return to normal conditions within 3 to 4 weeks after induction of anti-Thy-1 nephritis. The anti-Thy-1 nephritis model is therefore a typical example of a natural self-repair reaction after acute immune renal injury. However, the repair process is still poorly understood. Kriz *et al.* suggested that the injured glomerulus recovers as long as the disease

remains confined to the endocapillary compartment, and recovery seems to depend on the assembly of new capillaries in the injured glomerulus[58]. It seems that glomerular capillary repair occurs through the process of capillary regeneration from remaining endothelial cells as well as new glomerular capillary growth from the glomerular vascular poles. Among the players in the glomerular capillary repair process, vascular endothelial growth factor (VEGF)-stimulated capillary morphogenesis appears to take a leading role[59]. Furthermore, in glomerular capillary repair, apoptosis is necessary in regulating the number of intrinsic endothelial cells. Apoptosis is essential in the return of the glomerular structure to the original condition in anti-Thy-1 nephritis[60-62].

In general, anti-Thy-1 nephritis spontaneously resolves within several weeks, unless anti-Thy-1 antibody is repeatedly injected. However, in one particular sub-strain of Lewis rats, Lewis/Maastricht (Lew/Maa), after a single antibody injection the disease evolves to progressive glomerulosclerosis, while Lewis/Møllegard (Lew/Moll) rats spontaneously heal within 4 weeks[49]. For several reasons, the anti-Thy-1 nephritis model in combination with the two Lewis sub-strains provides a useful tool for assessing the pathogenesis and genetics of progressive glomerulosclerosis. First, a clear distinction can be made between repair and progression towards glomerulosclerosis after acute glomerular injury in a single model. Second, differences in disease development occur over a relatively short period of time (3 to 4 weeks). Third, the two Lewis sub-strains have small genetic differences, which limits the number of possible candidate genes involved in the repair- or progression process. Finally, because of the small genetic differences, organ transplantations from one sub-strain to the other sub-strain or vice versa can be performed without or with only a small risk of graft rejection. With organ transplantations, we can find the answer to the question of whether genetic susceptibility to progressive glomerulosclerosis in Lew/Maa rats results in intra- or extrarenal expression of the responsible genes. Intra- or extrarenal expression greatly determines the strategy of the following experiments and of possible therapeutic approaches.

Mediators in renal diseases: TGF-ß and decorin

Numerous studies describe cytokines as key factors in the development of renal diseases[63]. Cytokines are a group of proteinaceous signaling molecules that, like hormones and neurotransmitters, are used extensively for inter-cell communication. Cytokines are characterized by their complexity of actions, by means of their multi-functionality, their interactions with other cytokines, and their differential effects in different time frames. Various cytokines thought to be involved in renal diseases have been described, and one of these is transforming growth factor-beta (TGF-ß)[64]. TGF-ß belongs to the TGF-ß superfamily that

consists of more than 25 molecules, isolated from many species. The main sub-group includes three mammalian isoforms, TGF-ß1, -ß2 and -ß3. Of these, TGF-ß1 is most abundantly produced by mammals[65]. Mature TGF-ß molecules are synthesized and secreted as inactive pro-peptides (latent TGF-ß). After secretion, the latent molecules are proteolytically cleaved to the mature TGF-ß forms. It has been suggested that TGF-ß can bind to its own promoter, thereby inducing its own synthesis[66]. In addition, TGF-ß can also induce the synthesis of plasminogen activator inhibitor-1, which blocks the conversion of plasminogen into plasmin. Plasmin blocks the activation of the protease required for conversion of latent TGF-ß to active TGF-ß[67]. Various cell types produce TGF-ß, and the major sources are platelets, lung, bone, and placenta. The effects of TGF-ß are generated via binding of TGF-ß to TGF-ß receptor types I and II. TGF-ß possesses a wide and diverse range of biological actions, including control of cell proliferation, phenotypic changes, and adhesion. Furthermore, TGF-ß regulates, either positively or negatively, organogenesis, embryogenesis, inflammation, tissue repair, and fibrosis[68;69].

In the kidney, TGF-ß is present in normal glomeruli and in a variety of glomerular diseases[70]. Basal levels of TGF-ß may contribute to the maintenance of normal glomerular structure and function. TGF-ß promotes the production and deposition of ECM, which are essential both in physiological circumstances and in tissue repair following injury[71]. However, overproduction of TGF-ß leads to excessive production and accumulation of ECM molecules, resulting in scarring of renal tissue[72;73]. TGF-ß affects ECM turnover via inducing increased expression of ECM molecules, including various collagen molecules such as collagen types I, II, and IV, laminin, fibronectin, and glycoproteins, including osteopontin, osteonectin, biglycan, and decorin[71;74]. Furthermore, TGF-ß can inhibit matrix degradation by decreasing the activity of ECM-degrading matrix metalloproteases (MMPs) and by increasing the activity of tissue inhibitors of MMPs (TIMPs)[75;76]. Apart from regulating ECM turn-over, TGF-ß is also involved in many other processes, including modulation of the inflammatory response.

This pleiotropic character results from the several apparently opposing effects of TGF-ß. TGF-ß has been described as exerting both proinflammatory and anti-inflammatory effects. In the former role, TGF-ß enhances the attraction of inflammatory cells. In the latter, TGF-ß suppresses the inflammatory reaction during resolution of an immune response[77]. An essential role of TGF-ß in maintaining immunological homeostasis has been demonstrated in TGF-ß–deficient mice. These animals develop multifocal inflammatory diseases[78;79], while administration of exogenous TGF-ß in models of autoimmune diseases has been reported to result in immunoprotection[80]. Overall, TGF-ß is considered a key regulator in many different biological processes, including the generation of glomerulosclerosis. To better understand the pathogenesis of glomerulosclerosis, extensive efforts should be made to clarify the

pleiotropic character of TGF-ß[81].

Apart from inducing its own production, TGF-ß stimulates the synthesis of decorin, the natural antagonist of TGF-ß[74;82]. Decorin belongs to the class of glycoproteins, also known as proteoglycans, that are a major component of the ECM[83]. The proteoglycans are characterized by a common structure consisting of a core protein bound to one or more glycosaminoglycan side chains. A sub-family within the proteoglycan family is the group known as small leucine-rich proteoglycans (SLRPs). This sub-family differs from other proteoglycans in having a smaller molecular weight (on the order of 50 kDa). The primary structure of the core protein contains multiple repeat structures with a high leucine-rich content.

Decorin is a member of the SLRP family and carries a single glycosaminoglycan chain[84]. In the past decade, decorin has attracted considerable attention primarily because of its ability to affect several key biological processes. Decorin is known to modulate growth activity[82], tissue remodeling[85], collagen fibrillogenesis[86], receptor tyrosine kinase activity, angiogenesis[87], and bacterial infections[88]. One of the important characteristics of decorin is its binding to active TGF-ß, thereby neutralizing the activity of TGF-ß and permitting decorin to function as a reservoir of TGF-ß in the extracellular environment[89;90]. TGF-ß1, -ß2, and –ß3 are reported to bind to the decorin core protein with similar efficiency[91]. These findings suggest that decorin is the naturally occurring antagonist of TGF-ß[82]. The involvement of decorin in limiting TGF-ß1 activity has been suggested on the basis of the therapeutic effects of decorin administered to diseased rats, either by skeletal muscle-delivered gene therapy[92], mesangial cell-delivered gene therapy[93], or by recombinant protein[94]. In addition, absence of decorin adversely influences tubulointerstitial fibrosis of the obstructed kidney in decorin-deficient mice [95]. De Cosmo *et al.* reported allelic variance in the human decorin gene, which is related to slower progression of diabetic nephropathy in type 1 diabetic patients[96]. In human fibrosing renal diseases, decorin is present in the glomeruli, the tubulointerstitium, and urine[97-100]. Furthermore, it has been shown that expression of decorin in particular among ECM molecules predicts the severity of interstitial fibrosis and renal failure in a variety of glomerulonephritides[101].

To our knowledge, the anti-Thy-1 nephritis model in Lewis sub-strains is the only existing model in which a clear distinction can be made between repair and progression towards glomerulosclerosis after renal injury, whereas other models are focused on repair or on progression only. Investigating the actions of TGF-ß and decorin in the anti-Thy-1 nephritis model in Lewis sub-strains could provide a better understanding about how TGF-ß and decorin act in the process towards repair and progression following renal injury. In a previous study, we questioned whether the differences in disease development between the Lew/Moll

and Lew/Maa after anti-Thy-1 nephritis induction could be explained by differences in TGF-ß and decorin levels (de Heer *et al.*, unpublished data). Therefore, we measured glomerular TGF-ß and decorin protein levels during the first two weeks after induction of the disease. In the repair sub-strain (Lew/Moll), TGF-ß protein upregulation was accompanied by decorin accumulation, and these animals recovered spontaneously after a period of mesangial injury. In the progressive sub-strain (Lew/Maa), decorin expression lagged behind TGF-ß protein upregulation, and these animals developed progressive glomerulosclerosis. These data suggest that an inherited transient decorin deficiency is associated with progressive glomerulosclerosis after glomerular immune injury (de Heer *et al.*, unpublished data). With these results, new questions arose. First, can renal TGF-ß and decorin protein or mRNA levels determine the development of (human) renal diseases? Second, if reduced decorin levels are related to the development of a renal disease, could administration of decorin prevent the development of renal diseases? This thesis addresses both of these questions.

Aims of this thesis

The central aim of this thesis is to identify genes involved in the progression and repair of glomerular damage and to gain better insight into the pathogenesis of glomerulosclerosis. In the first studies described in this thesis, we focused on possible genetic factors involved in the process leading to the development of progressive glomerulosclerosis. **Chapter 2** presents an overview of genetic factors in progressive renal disease. Characterization of markers for genetic susceptibility to developing progressive glomerulosclerosis would be an important step towards prevention of progressive glomerulosclerosis. Furthermore, because of a lack of such markers, patients cannot currently receive an accurate prognosis for the development of progressive renal insufficiency. Comparing animal strains that are progressors with those that are not may circumvent these problems. Therefore, we studied the repair and progression of glomerular injury in the experimental renal disease model anti-Thy-1 nephritis is two rat sub-strains. The development of glomerulosclerosis in this model is the result of a dynamic interaction of the immune system and the kidney responding to inflammatory damage. The goal of this study was to determine whether in this model disease progression is mediated by genes intrinsic to the kidney, or by extrinsic factors. First, we exchanged bone marrow between the two Lewis sub-strains. Second, kidneys were exchanged between the sub-strains (**Chapter 3**). Then we performed a differential gene expression analysis using a rat cDNA microarray to identify genes involved in the development or repair of progressive glomerular injury following anti-Thy-1 nephritis. Time, duration, and localization of RNA and protein expression of the identified gene were established with realtime-PCR, immunohistochemistry, and double-

immunofluorescent staining (**Chapter 4**).

The studies described in the second part of this thesis were performed to contribute to the understanding of the pathogenesis of progressive glomerulosclerosis. Several studies have shown that sustained expression of TGF-ß in anti-Thy-1 nephritis is crucial for the subsequent development of glomerulosclerosis. Administration of decorin, a natural antagonist of TGF-ß, to rats with anti-Thy-1 nephritis has been shown to prevent the development of glomerulosclerosis. **Chapter 5** describes our investigations into whether either early or late administration of decorin to Lew/Maa rats during the induction of anti-Thy-1 nephritis has a therapeutic effect on the disease. Recombinant human decorin was injected intravenously at either an early or a late phase during development of the disease. In addition, we identified the TGF-ß–producing cells during the early and late phases of the development of anti-Thy-1 nephritis by double-label immunohistochemistry, *in situ* hybridization, and double-label immunoelectron microscopy. Finally, we hypothesized that differences in decorin expression between MCD and FSGS in the kidney may account for the differences between MCD and FSGS with respect to the development of glomerulosclerosis and tubulo-interstitial fibrosis. Renal biopsies from patients with MCD, FSGS, normal controls, and disease controls were immunohistochemically stained for decorin and TGF-ß. With real-time PCR and *in situ* hybridization, mRNA expression of decorin and TGF-ß were investigated in renal tissue samples from patients with MCD, FSGS, normal controls, and disease controls (**Chapter 6**). The results are summarized and discussed in **Chapter 7**.

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