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## **Proteinuria and function loss in native and transplanted kidneys**

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# General discussion

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The kidney is essential for the maintenance of the internal milieu of the body. Filtration of blood in the glomerulus is an important part of this process: plasma water is filtered, while most proteins and cells are retained. Damage to the glomerulus is often accompanied by proteinuria, the presence of abnormally high amounts of protein in the urine. Proteinuria presumably contributes to the progression of renal disease. At the tissue level, progressive loss of kidney function is mirrored by replacement of intact nephrons by scar tissue, a process called fibrosis. Once a critical amount of functional tissue is lost, the process of fibrosis is uncoupled from the initial cause of injury and becomes self-perpetuating.

Eventually, complete loss of kidney function will necessitate renal replacement therapy, for example through kidney transplantation. Also kidney grafts are subjected to injurious stimuli that drive the development of progressive loss of function. Peritransplant injuries, an immunologically hostile environment, and damage related to donor events contribute to development of progressive fibrosis in most renal allografts. Again, the pace of progression may be quickened by proteinuria.

In this thesis, we focused on two relatively distinct topics: the pathophysiology of proteinuria, and the recognition of causes of renal function loss in kidney transplants using a molecular approach. In this chapter, the results of the studies described in the previous chapters are summarized and placed in a broader perspective.

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## Proteinuria

A central but still incompletely answered question in renal physiology is how the glomerular filtration barrier works. Complementary to this is the question what the mechanisms of glomerular dysfunction are, in other words, what causes proteinuria. The different layers of the glomerular filtration barrier, the endothelium, the glomerular basement membrane (GBM), and the podocytes, are all assumed to contribute to the correct function of the glomerulus.

### Role of podocytes in proteinuria

In recent years there has been much interest in the role of the podocyte in the development of proteinuria. Indeed, podocytes show conspicuous changes during proteinuria: their normal complex shape is simplified through foot process effacement, and their delicate cytoskeleton is condensed at the basal part of the cell (1). This first morphological clue towards a role of podocyte changes in the development of proteinuria was further substantiated by the discovery of the genetic base of several congenital or hereditary forms of the nephrotic syndrome. Genes involved in the development of these diseases were found to be expressed exclusively in podocytes. This



led to the hypothesis that these genes and the proteins that they code for might also be involved in the development of acquired forms of proteinuria (2,3). Initial studies seemed to affirm this hypothesis: in acquired primary proteinuric diseases such as minimal change disease, FSGS, and membranous nephropathy, expression of the podocyte-protein nephrin was decreased (4-6). Animal studies also pointed towards a decrease in podocyte-associated proteins in proteinuria (7,8). However, most of these studies described the expression of single molecules, and only studied either mRNA or protein level. We wanted to further investigate the expression of a group of podocyte-associated molecules and relate the expression to ultrastructural and clinical parameters. This was the subject of a study described in chapter 2. We studied the glomerular mRNA and protein expression of several podocyte-associated molecules in patients with acquired nephrotic syndrome, in comparison to that of patients with a non-glomerular type of kidney disease and healthy controls. In line with previous studies, we indeed found a decrease in podocyte proteins in proteinuric diseases. At the same time, our results suggested that the loss of protein expression is not the actual cause of proteinuria: comparison of protein expression levels with the podocyte ultrastructure made clear that the decreased protein staining could be explained by the loss of the specialized podocyte architecture. Also, mRNA synthesis was not impaired, but was even increased in most diseases, suggesting a compensatory reaction of the podocyte to proteinuria.

A shortcoming of the study presented in chapter 2 may be that support for the hypothesis, that the podocytes show a compensatory reaction to proteinuric damage, is merely derived from correlations rather than from mechanistic studies. Furthermore, most cases of human acquired proteinuric diseases already showed high levels of proteinuria at the time the renal biopsy was taken. This limited the evaluation of changes in podocyte ultrastructure and mRNA and protein expression in time. To gain insight in the processes at play in the podocyte during the development of proteinuria, we used a rat model of proteinuria development, the Dahl salt-sensitive (Dahl SS) rat. These animals spontaneously develop proteinuria starting from week 4 to 6 of age, and later on show progression to glomerulosclerosis. We were interested in the early phases of proteinuria, and studied these rats at different time points between their 2nd and 10th week of life. The results of the studies in rats, outlined in chapter 3, were in line with the concept raised from the study in patients, ie, the changes in expression of podocyte-associated molecules do not precede proteinuria. Instead, despite clear disturbance in glomerular permselectivity as measured by urinary albumin and protein excretion at ten weeks of age, expression of most podocyte-associated molecules was still normal at that time, except for some segmental loss of nephrin staining intensity. Podocyte ultrastructural changes, most importantly foot process effacement, also notably lagged behind the development of proteinuria.

The one exception to this general pattern of undisturbed protein expression of studied podocyte-associated molecules was the expression of the glycoprotein podoplanin. We observed a segmental loss of podoplanin expression already at four weeks of age – the first time point at

which proteinuria could be detected. Others have already related the expression of podoplanin to proteinuria. In fact, the name podoplanin has been derived from the fact that loss of this protein occurs simultaneously with foot process effacement (*pes planus* (lat.) – flat feet). Also, injection of anti-podoplanin antibodies in rats induces proteinuria (9-11). In our study, the decrease in expression of podoplanin in proteinuric rats compared to non-proteinuric rats was only seen at the protein level. There were no genetic differences between Dahl SS and SHR rats in the podoplanin gene that could explain proteinuria, and podoplanin mRNA expression was even increased in Dahl SS rats compared to SHR rats. Furthermore, glomeruli from proteinuric Dahl SS rats with and without podoplanin loss showed similar podoplanin mRNA expression levels.

Next to expression in the glomerular podocyte, podoplanin is found in several other cells, including alveolar cells, osteoblasts, skeletal muscle, and lymphatic endothelial cells (12). Also, a number of tumors show expression of podoplanin. Several investigators have described that podoplanin is coupled to ezrin, which connects podoplanin to the actin cytoskeleton (13,14). Ectopic expression podoplanin in cultured cells induces the formation of filopodia (actin-supported cell extensions), a finding that would explain the migratory behavior of podoplanin-positive tumor cells (15). Podoplanin exerted this actin-remodeling effect via downregulation of the activity of RhoA, a member of the Rho family of GTPases that are involved in the (dis)assembly of actin filaments. Whether podoplanin has a similar role in podocytes has not been established yet. We did not find differences in ezrin mRNA expression, but this does not rule out a difference in ezrin-podoplanin interaction at the level of post-translational protein modifications. With respect to actin-remodeling properties of podoplanin, we did find a trend towards upregulation of RhoA mRNA expression in podoplanin-negative glomeruli. This suggests a link between podoplanin expression and actin cytoskeleton regulation. In analogy to the role of podoplanin in filopodia formation via RhoA downregulation, we hypothesize that a decrease of podoplanin protein expression in the podocytes facilitates RhoA-mediated foot process effacement and actin cytoskeleton condensation. Further evidence that may confirm or disprove such theories will have to come from in-vivo manipulation of podoplanin, for example through genetic approaches. Podoplanin knock-out mice die of respiratory failure soon after birth (16). Kidney defects or maldevelopment in these mice have not been studied. Investigations into the role of podoplanin in the podocyte will benefit from mouse models with a podocyte-specific podoplanin deletion. The Cre-loxP system for podocyte-specific gene manipulation is available (17,18), but mice with podoplanin-floxed alleles have not been generated yet. It would be of interest to see whether such podocyte-specific podoplanin deficient mice develop a normal glomerulus, and whether they show proteinuria or increased disease susceptibility.



## Glomerular gene expression in proteinuria

Besides our study of the expression of well-known podocyte-associated molecules, we also evaluated proteinuria-associated changes in glomerular mRNA expression through a more genome-wide approach. The results of these experiments are described in chapter 4. We used microarray chips to which we hybridized RNA isolated from glomeruli of 4- and 6-week-old Dahl SS and SHR rats. These time points were chosen because they flank the period in which the first onset of proteinuria is seen.

For the evaluation of the array results we used several approaches. In recent years, the labs of Kreutz et al (19-21) and Garrett et al (22,23) have identified quantitative trait loci (QTLs) that are linked to the development of albuminuria in the Dahl SS rat. We evaluated which of the differentially expressed genes between the two rat strains localized to these genome regions. Secondly, we used pathway analysis software to evaluate which pathways were represented in the list of differentially expressed genes. Furthermore, we compiled a list of genes known to be differentially regulated upon protein loading in renal proximal tubular epithelial cells, and evaluated the glomerular expression of these genes using the global test (24).

We identified around 500 genes that were differentially regulated between the two strains, regardless of the time point. Of these, 115 genes were located on the previously defined QTLs. We validated the expression of hedgehog interacting protein (Hip, 5.2 times upregulated), and polyamine modulating factor binding protein 1 (Pmfbp1, -2.8 times downregulated). Both genes are located on the albuminuria QTL on rat chromosome 19.

Pathway analysis indicated an overrepresentation of cytoskeleton-associated genes in the list of differentially expressed genes. This may be explained by the fact that proteinuria is known to occur simultaneously with changes in the cytoskeletal organization of podocytes. During the first 8 weeks of life of the rats we did not observe widespread differences in the ultrastructure of the podocyte foot processes, meaning that already early in the development of proteinuria there is a change in podocyte cytoskeleton regulation that is not reflected in morphological alterations. We studied the GTPase dynamin in more detail. Recent studies by the Reiser group have indicated a role for dynamin in the organization of the podocyte actin cytoskeleton (25). In their studies, a cathepsin L-dependent cleavage of dynamin resulted in proteinuria. Our results contrast their findings, as we found an increase in dynamin mRNA expression in proteinuric rats, as well as an increased dynamin protein expression in proteinuric patients. The models in which dynamin was studied differ considerably between our study and that from the group of Reiser. We used a model of spontaneous development of proteinuria, while in the animals studied by Reiser and colleagues, proteinuria was induced by LPS or puromycin aminonucleoside. This may indicate that the regulation of dynamin in diverse proteinuric conditions is different. Similar to the concept

discussed in chapter 2, the upregulation of dynamin may reflect a compensatory response to the development of proteinuria.

Furthermore, we found that a group of genes that is differentially expressed upon protein loading in proximal tubular epithelial cells is also differentially regulated in glomeruli of proteinuric versus non-proteinuric rats. This finding substantiates the hypothesis that increased glomerular protein filtration has a toxic effect on the podocyte. In support of this, a double staining of albumin and desmin (a podocyte stress marker) showed a colocalization of albumin resorption droplets and desmin accumulation in the podocytes.

## Further perspectives on proteinuria

### Development of proteinuria

In trying to delineate the pathogenesis of proteinuria, the problem is that regardless which component of the glomerular filtration barrier is damaged the result is proteinuria. This shows that the different components of the glomerular filtration barrier are all indispensable, or are functionally interlinked. This functional relationship is obvious. For example, podocytes and endothelial cells produce the GBM they rest on; malfunction of either of these cell types may lead to a disturbed glomerular filtration that is difficult to trace back to a single cause. Adding more complexity to the situation is the fact that the filter may only work properly if not only the static components are correctly assembled, but also the glomerulus is perfused under physiologic conditions, as this may lead to the *in vivo* formation of pressure and charge gradients that have a role in glomerular permselectivity. This complex interrelationship between different static and functional components of the filtration barrier makes it difficult to establish their respective roles in glomerular filtration in health and disease. Thus, the question at which site glomerular filtration takes place, posed more than 30 years ago by Farquhar in a review on the topic (26), is at present still valid. Still, while important parts of the pathogenesis of proteinuria remain uncharted, some pathways can be delineated. The perspectives on the different components of the glomerular filtration barrier will be discussed below.

#### *Interplay between proteinuria and foot process effacement*

Podocytes are involved in every type of glomerular proteinuria. Clearly, in congenital proteinuric diseases podocyte damage is often the cause of proteinuria (see chapter 1). However, the studies in this thesis suggest a secondary role for podocytes in acquired proteinuric diseases: Foot process effacement only developed after the occurrence of proteinuria (chapter 3); podocyte protein expression remained normal in the initial stages of proteinuria (chapter 3), changes in expression of most podocyte-associated proteins (except that of podoplanin in the Dahl SS rat) seemed to be related to the extent of foot process effacement (chapter 2, 3). Also, a selected number of genes



that are expressed in tubular epithelial cells as a consequence of proteinuria also show up in the glomerular gene expression profile in proteinuric rats (chapter 4).

Several studies in the literature support the above findings. Some authors have argued that foot process effacement affects the process of glomerular filtration in such a way that proteinuria occurs (27), ie, foot process effacement is the cause of proteinuria. However, most recent studies are compatible with foot process effacement as a result of proteinuria. Jarad et al (28) found that proteinuria preceded podocyte foot process effacement in a podocyte specific laminin  $\beta 2$  knock-out mouse model. Kalluri described three different mouse models that show proteinuria without initial foot process effacement (29). Van den Berg et al have found that foot process effacement is not related to the amount of proteinuria in minimal change disease: patients in remission showed a foot process width that was comparable to that in patients with overt proteinuria (30).

#### *Possible causes and consequences of foot process effacement*

The exact cause of foot process effacement remains unclear. Also, the role of this process in glomerular function is not completely understood. Dysfunctional podocyte proteins, changes in GBM composition, protein overload, and (lack of) stimulation by growth factors and cytokines have all been related to the development of foot process effacement. Clearly, this is a final common pathway of podocyte damage.

From a teleological point of view, several interesting hypotheses have been raised to explain what goal foot process effacement could serve: Reiser et al found that podocytes express the co-stimulatory molecule CD80. Increased expression of CD80 was found in conjunction with the development of proteinuria. This suggested to them that podocytes might have a role in immunological danger signaling, and display a stereotypical reaction to such 'danger signals'. In a review on this novel aspect of podocyte function, Reiser and Mundel suggested that foot process effacement and the resulting increase in glomerular permeability could have a role in increasing the speed with which noxious molecules are cleared from the blood (31,32). However, it is questionable whether this theory is valid in situations where, as outlined above, foot process effacement follows the development of proteinuria.

Another interesting theory (33) suggests that foot process effacement is a way for the glomerulus to counteract the increased intracapillary pressure. The different shape of the podocyte, with the reinforced cytoskeleton, would then increase stability at the expense of permselectivity.

#### *The role of the GBM and the endothelium*

The glomerular basement membrane supports the endothelial cells and podocytes. The GBM per se seems to have only a minor role in glomerular filtration, although it is indispensable for the correct function of podocytes and endothelial cells. For example, patients with Pierson's syndrome, caused by a mutation in the laminin  $\beta 2$  gene, are proteinuric and show foot process effacement.



As discussed in chapter 1, the glomerular filtration barrier is size- and charge selective (34). With regard to the charge selectivity, several recent studies have excluded an important contribution of heparan sulphate proteoglycans (HSPGs) in the GBM to charge selectivity (35-37).

If HSPGs do not contribute to the charge selectivity, what role do they serve? In embryogenesis, HSPGs have a role in distribution and gradient formation of signaling molecules such as hedgehogs. One could speculate that in a similar fashion HSPGs in the GBM have a role in the interaction between endothelial cells and podocytes. Indeed, such interaction may be important in glomerular filtration: Collino et al have recently shown that stimulation of endothelial cells with plasma of women with pre-eclampsia releases factors that subsequently influence podocyte homeostasis (38).

Recent studies have shown that the endothelial fenestrations, traditionally thought to be too large to contribute to ultrafiltration, may be filled with proteoglycans that could contribute to the charge and size selectivity of the glomerulus. With this in mind, it is to be expected that the glomerular endothelium has a more important role in glomerular function and dysfunction than hitherto acknowledged. A pivotal role of endothelial cells would provide a plausible explanation for the proteinuria that is seen in diseases with systemic endothelial dysfunction, such as diabetes and pre-eclampsia. Furthermore, it would explain the observation that proteinuria is a risk factor for the development of cardiovascular diseases, as both diseases can be traced back to endothelial dysfunction (39).

If indeed the endothelium is an important first barrier to protein, this would mean that in a normal situation the GBM and podocytes are not exposed to plasma-concentrations of protein. Loss of endothelial function would lead to a higher concentration of protein in the GBM and thus at the basal cell membrane of the podocyte foot processes. From this perspective, our observation of a proteinuria-related gene response in the glomerulus (chapter 4) would be compatible with the hypothesis that size selectivity of the glomerulus is located in the endothelium. Indeed, when we directly stained frozen slides of Dahl SS and SHR rats with anti-rat-IgG antibodies, we found an increased distribution of rat IgG in the GBM and podocytes of the proteinuric Dahl SS rat (results not shown). This is in line with observations in humans with proteinuric kidney diseases (40). In this regard, it is of interest that the Dahl SS rat later develops hypertension, which has been regarded as another symptom of endothelial dysfunction (41). Thus, future studies should focus on the role of the endothelium in the glomerular filtration barrier. There have been reports of genes expressed specifically in glomerular endothelium (42). This offers opportunities to generate transgenic mouse models that can be used to dissect the role of the endothelium in glomerular filtration.



### Consequences of proteinuria

While the cause of proteinuria remains unclear, the consequences of proteinuria are more clear, at least from an epidemiologic point of view: proteinuria is associated with the progression of renal disease. This raises the question whether proteinuria solely reflects the damage inflicted upon the nephron, which leads to further progression of renal disease, or that proteinuria forms a part of the pathogenetic process that underlies progression of renal disease.

As discussed in chapter 1, reports from the literature on this topic are controversial: some studies bring support for the possibility that proteinuria causes tubulointerstitial inflammation and fibrosis, others dispute such a link. In either scenario, impaired glomerular function precedes the development of progressive renal disease. Our findings (chapter 4) are compatible with the hypothesis that proteinuria is part of the pathogenetic process that leads to further loss of glomerular function, as judged from gene-expression patterns and histological studies. Others have described TGF- $\beta$  and endothelin upregulation in podocytes in response to proteinuria (43,44), also pointing towards a deleterious role of proteinuria in glomerular function.

Once a critical amount of cells and tissue are damaged, loss of glomerular function becomes self-perpetuating. This vicious cycle seems to be present at two levels in the kidney: At the tissue level, loss of nephrons increases the workload for the remaining nephrons, leading to hyperfiltration and further damage. At the cellular level, loss of podocytes increases the demands placed on the remaining cells. Wiggins et al described a sequence of podocyte hypertrophy, adaptation, and decompensation in rats with a relative shortage of podocytes (45). The stress placed on podocytes as a result of increased protein trafficking through the glomerulus may be the driving force for this process.

## Chronic allograft dysfunction

There has been a clear progress in renal transplant survival rate over the years. Especially the first year graft-survival rate has increased tremendously through improvements in immunosuppressive medication, and monitoring of its administration. Nowadays, failure of renal grafts on the long term is the most limiting factor in transplantation.

Multiple causes may underlie the development of chronic allograft dysfunction, but the eventual course is common with respect to clinical and histopathological presentation: there is a slow decline in renal function, mirrored at the histopathological level by the presence of vascular damage, tubular atrophy, and interstitial fibrosis. Different causes of chronic allograft dysfunction often coexist, thus forming a spectrum of injurious stimuli ranging from the toxicity of excess immunosuppressive medications to an ongoing activity of the immune system. An important question is how to distinguish the separate causes in patients that present with non-specific clinical and histopathological features. The studies described in chapters 5 and 6 aimed to address this question using molecular techniques. In chapter 5, we studied the mRNA expression levels of growth factors and extracellular matrix (ECM) proteins that are important in the development of interstitial fibrosis. In chapter 6, we used an immunohistochemical analysis of the composition of interstitial fibrosis. These markers were studied using biopsies from renal allografts of patients from two well-defined groups, each representing one side of the spectrum of different causes of chronic allograft dysfunction: the chronic rejection group and the chronic cyclosporine A toxicity group.

The composition of the ECM is continuously modified through synthesis and degradation. Previous experiments have shown that pathophysiological changes could be observed more readily at the level of synthesis, through measurement of mRNA expression levels of molecules known to be involved in the disease process. As described in chapter 5, differences in mRNA expression of laminin  $\beta 2$  and TGF- $\beta$  could differentiate between chronic rejection and chronic cyclosporine A toxicity as a cause of chronic allograft dysfunction. The mRNA expression levels of these proteins were higher in the cyclosporine toxicity group than the chronic rejection group. Analysis of laminin  $\beta 2$  and TGF- $\beta$  mRNA expression levels allows recognition of the actual cause of allograft dysfunction with a specificity and a sensitivity of over 85 percent.

In chapter 6, we have shown that the accumulation of collagen I in interstitial fibrotic lesions was more pronounced in patients with chronic rejection than that in patients with chronic cyclosporine toxicity. Collagen III and IV accumulated in the interstitial area to similar extent in either disease. Others have found that an ectopic production of certain collagen and laminin chains



could help differentiate chronic rejection and cyclosporine A toxicity (46). In our studies, we could not confirm that such abnormal expression patterns can distinguish between different disease entities.

Besides showing that molecular techniques can have additive value in dissecting the cause of clinically non-specific disease processes, the studies in chapter 5 and 6 may give insight in pathophysiological processes that play a role in transplant rejection. For example, from the results of the studies described in chapter 6, we propose that chronic rejection seems to stimulate the accumulation of collagen I more pronounced in comparison to chronic cyclosporine toxicity. However, the mRNA expression of collagen I was similar in both diseases, suggesting a role for an impaired collagen I degradation process in chronic rejection. The increased laminin  $\beta$ 2 mRNA expression in chronic cyclosporine A toxicity might point to a stimulatory effect of cyclosporine A production on the synthesis of this transcript. Since vascular damage may stimulate laminin  $\beta$ 2 production, the increase in laminin  $\beta$ 2 expression could also reflect a more pronounced vascular damage in cyclosporine A toxicity compared to chronic rejection. It should be stated, however, that such pathophysiological hypotheses are based only on correlative evidence.

An interesting finding in these studies was that increased mRNA expression does not consistently coincide with protein accumulation. Besides differences in protein degradation, changes in mRNA processing and regulation as well as variations in the efficiency of mRNA to protein translation may account for these differences. It has been shown that even in unicellular eukaryotic organisms such as yeast there is no direct correlation between mRNA expression and protein abundance (47). This again underscores the complexity of regulation of processes such as fibrosis, and indicates that some caution in extrapolating result between different levels of organization (i.e. mRNA, protein, cellular, and tissue level) is warranted.

The studies described in chapters 5 and 6 were performed in two well-selected populations of chronic cyclosporine A toxicity and chronic rejection, representing both ends of the spectrum of chronic allograft dysfunction. Although this was helpful in defining a molecular profile that could distinguish both causes, the strict selection of patients also forms the main limitation of the study. As stated before, in many patients, different causes of allograft dysfunction coexist, and therefore we cannot draw conclusions with regard to the usefulness of these markers in non-selected patient groups. In the future, the markers studied by us and by others should be tested in larger, unselected patient groups.

## Further perspectives on molecular diagnosis of chronic allograft dysfunction

What is to be expected from future research of molecular markers in chronic allograft dysfunction? The search for markers that can identify specific causes of allograft dysfunction, or distinguish progressors from non-progressors in an early phase of the disease, will be instrumental in better treatment of patients with a renal transplant. Also, further knowledge of the precise pathogenesis of allograft dysfunction will be needed to improve the transplant survival rate. Use of epidemiologic data may be helpful in selecting which pathogenetic processes to study. These topics will be discussed below.

Microarray studies would seem to be a logical next step in the search for markers of specific causes and mechanisms of chronic allograft dysfunction. Several microarray studies have already been reported, which have offered important new information with regard to pathophysiological processes in acute rejection (48,49). For example, the role of humoral rejection became more apparent in the microarray-based analysis of acute rejection biopsies. However, microarray-assisted investigations in chronic allograft dysfunction have so far not brought up new pathophysiological concepts. Hotchkiss et al (50) studied expression profiles in biopsies from patients with chronic allograft dysfunction with and without arteriolar hyalinosis (suggesting a different pathogenesis), but could not find a different expression profile, most likely because the disease had already passed to a chronic phase or final common pathway, in which specific causes are obscured by a general process of renal deterioration. This shows that molecular markers that identify specific causes of allograft dysfunction, or distinguish progressors from non-progressors should be found early in the disease process, when specific therapies can still be installed. This leads to another problem: a relatively long follow-up time is required to determine early markers that reliably predict the long-term outcome. During this long follow-up time, immunosuppressive regimens may change, and as a result the markers found in these long-term studies may not be applicable for the next 'generation' of patients. For example, the studies described in chapters 5 and 6 included only a few patients that used tacrolimus, while at the moment most transplant centers have switched from cyclosporine to tacrolimus as a calcineurin inhibitor. Thus, frequent changes in immunosuppressive regimes will impede the evaluation of long-term effects of medication, and even more, the evaluation of the usefulness of early molecular markers for long-term prediction of transplant course.

Instead of focusing on the specific characteristics of processes leading to chronic allograft dysfunction, another fruitful strategy could be to identify the pathophysiological processes that chronic allograft dysfunction shares with other chronic kidney diseases. Again using microarray, Donauer et al found that the expression profiles of native end-stage renal disease kidneys were similar to those of transplanted kidneys with chronic allograft dysfunction (51). Also, risk factor



analysis makes clear that not only immunological mechanisms but also non-immunological injuries are of importance for long-term graft survival. Donor age, for example, is one of the most important risk factors, accounting for approximately 30 percent of the variation in long-term graft survival (52). Together, these microarray experiments and epidemiological data may indicate that the pathophysiologic mechanisms that drive the deterioration of kidney grafts are similar to those at play in progressive disease of native kidneys. A more extensive comparison of both would enhance the insight in the nature of disease progression.

### **Other lines of investigation**

Because the kidney is transplanted into an immunologically hostile environment, one is inclined to think that the processes leading to transplant failure are predominantly immunological in nature. The notion that native and transplanted kidneys follow similar final pathways to renal function loss shows that this is not necessarily the case, and also illustrates the importance of the use of epidemiologic data. In this regard, it is interesting to note that there are considerable differences in the reported kidney allograft half-life between continents. In the USA, reported 10-year renal allograft survival rates amount to about 40 percent, while in France, 10-year survival rates of 61 percent were reported (53). Also the allograft survival in the Netherlands is considerably longer than that in the USA (M. Mallat and Y. Sijkens, personal communication). It would be interesting to compare on an international level the risk factors of chronic allograft dysfunction in order to explain this divergence of long-term survival rates.

As outlined previously, proteinuria is a risk factor for progression of renal disease in both native and transplanted kidneys. Because proteinuria, in contrast to risk factors such as donor age, may be to some extent modifiable, it would be interesting to study the role of proteinuria in the progression of allograft dysfunction. Post-transplant proteinuria may be caused by certain immunosuppressive drugs (54), and also in the setting of post transplant glomerulopathy. The latter is a specific pathophysiological process that is thought to mainly involve humoral rejection (see chapter 1), primarily resulting in endothelial damage (55). The proteinuria that is seen in most cases of chronic allograft dysfunction is not necessarily caused by immunological mechanisms. Instead, more general mechanisms that are also involved in native glomerular disease and ageing may be at play. The glomerular damage seen with increased age is thought to be a podocyte disease: as a result of several stress factors, podocytes are lost and cannot be replaced. This is suggested to be the starting point of a final common pathway to glomerulosclerosis, and via pathways lined out in the introductory chapter on nephron loss (56). Prevention of age-related podocyte damage may in part be accomplished by reducing hypertension, preferably with angiotensin II blockers that have a kidney- or podocyte-protective effect. Similar to the 'accelerated senescence' concept proposed by Halloran to explain pathophysiologic processes in transplanted kidneys, glomerular aging may also progress faster in transplantation. Attempts to limit other podocyte-damaging

factors, for example through blood pressure control using angiotensin II blockers, may thus prove beneficial in native and transplanted kidneys alike.

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