Immune-mediated podocyte injury and the idiopathic nephrotic syndrome
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Chapter 7

General Discussion
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Nephrotic syndrome (NS) is characterized by massive proteinuria, hypoalbuminemia and edema. The occurrence of massive proteinuria is usually, yet not always, associated with effacement of the podocyte foot processes. Recent advances in the field of molecular biology have enabled the elucidation of the causes of various familial forms of NS. Concomitantly this has provided a wealth of information on the molecular make-up of the glomerular filtration barrier. In contrast to these rapid advances in the field of hereditary NS, our insight in the pathogenesis of non-familial NS is still limited. As outlined in Chapter 1, proteinuria and NS can be induced by several types of podocyte injury, including intrinsic and extrinsic damaging factors. Familial NS is often caused by intrinsic defects in the glomerular filtration barrier, due to mutations that for instance encode podocyte proteins involved in cell signaling, cell-matrix or cell-cell contact. In contrast, many studies suggest that non-familial NS may result from extrinsic damage caused by a circulating factor.

The aim of our study was to obtain more insight in the pathogenesis of minimal change nephrotic syndrome (MCNS), the commonest form of NS in children and mainly occurring in a non-familial setting. More insight in its pathogenesis could promote the development of more specific drugs that could replace the current treatment with corticosteroids. This thesis was based on two basic hypotheses. First, in analogy to its central role in hereditary NS, we assumed that the podocyte would play a pivotal role in the pathogenesis of MCNS. Second, we hypothesized that cytokines may represent soluble circulating permeability factors that could exert direct actions on podocytes and thereby play a role in the pathogenesis of MCNS.

We mainly focused on the role of Type2 cytokines, because of the reported association of NS with atopy (for references see Chapter 1). Despite the multitude of reports, it should be remembered that this association is difficult to prove, since (1) here is no standard definition of the entity "atopy", (2) the prevalence of atopy varies between populations and countries, and therefore there is no official number on the prevalence of "atopy" in the general pediatric population, and (3) the incidence of allergic reactions in children is high. Still, many experimental studies have reported on an altered immune balance in patients with MCNS, with a cytokine bias towards Type2 reactions (for references see Chapter 1). In Chapter 4, we studied the expression of various Type1 and Type2 cytokines by PBMC from patients with MCNS during relapse and remission by quantitative real-time PCR. In contrast to many studies performed in the past, we included only patients that did not use immunosuppressive drugs. Furthermore, we included a control group of patients with NS primarily caused by endogenous alterations within the glomerular filter. Of the cytokines studied, only the expression of IL-10 and IL-13 mRNA was significantly up-regulated in relapsing MCNS patients, when compared with patients with MCNS in remission. The expression of IL-13 mRNA, however, was also upregulated in the nephrotic control group. In the nephrotic state the composition of circulating blood changes, with hyperlipidemia as a common complication. This altered state itself may activate the immune system, as shown by a study of Lenarsky et al. who reported that the presumed immunosuppressive effect of serum of nephrotic patients, which had been claimed before, could be simply reversed by removal of the lipoprotein fraction [1]. Thus, hyperlipidemia itself modulates the immune system and may alter also the production of circulating factors, which may be easily misinterpreted as permeability factors. In our study, the expression of IL-13 appeared to be related to the nephrotic state, rather than specifically associated with MCNS.

In patients with MCNS, the glomeruli show no significant influx of inflammatory cells and no signs of immune complex deposition. Therefore, the activation state of T cells in nephrotic patients, as described above, thus does not seem to result in inflammatory events at the glomerular capillary wall. However, cytokines and other circulating factors may be able to directly act on the glomerular capillary wall. We hypothesized that these interactions may take place via cell surface receptors.

The podocyte is equipped with cell surface receptors at the basal cell membrane. These receptors include alpha 3 beta 1 integrin with CD151 and integrin linked kinase [2-4], and the dystroglycan complex [5, 6]. These adhesion receptor complexes are linked to the
submembranous actin cytoskeleton, and mediate not only cell adhesion, but also inside-out and outside-in signaling. In Chapters 2 and 4 we show that podocytes constitutively express functional transmembrane receptor complexes for Type2 cytokines IL-4, IL-10 and IL-13, which signal upon exposure to their cognate ligands. The IL-4/IL-13 receptor complex is also expressed by endothelial cells, including glomerular endothelium in vivo (Chapter 2 and [7]). Parry et al. confirmed that podocytes express receptors for IL-4, IL-10 and IL-13 [8], and in another study, we have shown that podocytes also express receptors for CD134 and TNF-α [9]. The presence of cytokine receptors at the glomerular capillary wall is puzzling and raises teleological questions: what is their significance in normal states and what is their role in disease?

In immune cells, cytokines function as soluble messengers and binding of cytokines to their cognate receptors induces a variety of intracellular changes, which have been studied extensively. These changes include cytoskeletal rearrangements, as has been shown for IL-4 and IL-13 in B cells, granulocytes and macrophages [10-14]. Also in cultured human umbilical vein endothelial cells, IL-4 and IL-13 regulate cell morphology, cytoskeleton and proliferation [15]. In Chapter 3, we show that in cultured podocytes, IL-4 and IL-13 directly alter protein sorting and ion transport. We found that IL-4 and IL-13 promote secretion of protons towards the basal surface of the podocytes, thus creating an acidic environment. This would promote local activity of lysosomal enzymes that are produced and secreted by podocytes, such as cathepsin L (Chapter 3) and heparanase (Chapter 5), towards matrix constituents.

Heparanase is an enzyme that specifically cleaves heparan sulfate (HS). In Chapter 5, we show that the expression of heparanase is increased in glomeruli of MCNS patients, which is associated with decreased presence of HS in the GBM. We speculated that by means of breakdown of HS side chains of agrin, heparanase would largely alter the function of agrin in the GBM. Agrin not only contributes substantially to the anionic charge of the GBM, but also plays an important role in the assembly and aggregation of other GBM macromolecules and growth factors. Furthermore, it could play a role in the regulation of cell signaling in the podocyte. At the neuromuscular junction, certain motor neuron-derived agrin variants are required for activation of muscle-specific kinase and thereby for MuSK-dependent synaps organization [16]. In T cells, agrin mediates cell signaling by lipid rafts and thereby plays a role in the organization of T cell polarity [17]. Interestingly, this action is mediated by a specific active form of agrin with low molecular weight, which is probably a low sulfated form of agrin that may be generated by heparanase [18]. A similar role for glomerular agrin in the regulation of podocyte signaling by lipid rafts deserves further investigation. It would have important implications for the contribution of agrin and heparanase to the maintenance of podocyte foot processes, since the slit diaphragm components nephrin, podocin, NEPH1, P-cadherin and FAT are all organized in lipid rafts [19].

In the work presented here, we did not observe direct actions of Type2 cytokines on podocyte cytoskeleton that could be directly related to podocyte foot process effacement. However, our studies were hampered by the fact that the cultured podocytes used are cells that in vitro do not form foot processes and do not express slit diaphragm components. The recent generation of human cultured podocytes that do display foot processes [20] could provide a useful tool to study effects of cytokines on podocyte architecture in vitro in the near future, with special attention to the cytoskeleton and the mechanisms mentioned above.

It is particularly difficult to translate work in vitro to the situation in vivo, since the glomerular capillary filter is a complex structure with multiple interactions that cannot be studied in vitro. The effect of IL-4 on the glomerular capillary wall in vivo was elegantly shown in two successive studies on IL-4 transgenic mice. Transgenic mice that overexpressed IL-4 under the control of an MHC class I promoter developed glomerular FSGS-like lesions, associated with proteinuria [21]. This phenotype was associated with increased production of auto-antibodies and the presence of immunoglobulin deposits in the glomerular filter, and renal disease could be prevented by treatment with IL-4 neutralizing antibodies. In a crossbreeding experiment with these mice and with μ-chain-deficient mice (μMT-/-), IL-4 transgenic mice were generated that were unable to produce immunoglobulins. In the absence of glomerular depositions, these mice still displayed FSGS-like lesions and proteinuria [22]. Possibly, in these mice direct effects of IL-4 on the glomerular capillary...
wall via the IL-4 receptor were involved in the development of disease. In contrast to IL-4, overexpression of IL-13 in transgenic mice under control of the CD2 promoter [23] did not induce glomerular abnormalities or proteinuria [unpublished data].

Thus, cytokine receptors render the glomerular capillary wall responsive to immunological signals from the environment. One may hypothesize that under physiological circumstances the levels of circulating cytokines are low and their effects at the glomerular capillary wall may be less pronounced, whereas in disease, elevated cytokine levels may induce several changes and eventually lead to an increased glomerular permeability to protein. Alternatively, binding of cytokines to their receptors at the glomerular capillary wall may stimulate the production of other factors by endothelial cells and/or podocytes, which in turn may function as permeability factors in a paracrine or autocrine manner.

The actual mechanism that causes proteinuria in any model that has been studied thusfar, is still unclear. Also it is still indefinite how the kidney in the normal state in fact prevents loss of proteins into the urine. For years it has been assumed that the glomerular capillary wall imposes a physical barrier for proteins. However, at which level this barrier would be located, i.e. endothelium, GBM and/or podocyte, is still a matter of debate. Moreover, it is unclear why the glomerular capillary filter does not become obstructed if proteins enter the filter at a continuous rate and subsequently are captured in its meshes. How the capillary filter functions in the case of podocyte foot process effacement is even more obscure.

In Chapter 6, we conducted a morphometrical study on the relationship between podocyte foot process effacement and proteinuria. In accordance with some earlier reports in the literature, we showed that podocyte foot process effacement and proteinuria are not always related, and concluded that foot process effacement is not a prerequisite for development of proteinuria.

Recently, some workers disputed the classical concept of a semi-permeable glomerular filter, and postulated that by diffusion the glomerular filtration barrier would be permeable to proteins [24-27]. In this concept, proteins in the filtrate would be retrieved by the proximal tubular cells. In the case of podocyte foot process effacement or a fall of glomerular filtration rate, the concentration of albumin achieved in the tubules would be increased and thereby the retrieval of albumin would become saturated, resulting in proteinuria. This model, as proposed by Smithies [27], would explain "why the kidney glomerulus does not clog", as well as the possibility of proteinuria in the absence of podocyte foot process effacement. However, the existence of massive albumin retrieval in the proximal tubules is subject to discussion, since such retrieval is thought to be accompanied by massive breakdown and thus loss of albumin. Still, in both concepts, many questions on the glomerular filtration barrier and proteinuria remain unanswered, and re-evaluation and integration of new, current and old models is needed.

Altogether, our study provides a possible link between the clinical association of MCNS with allergic events and the functional and structural changes in the glomerulus during MCNS. We have shown that Type2 cytokines can directly act on podocytes and the glomerular filtration barrier, yet the action of a single cytokine may not be sufficient to induce disease. The finding that only few allergic patients develop NS, while they all show high levels of circulating Type2 cytokines, would suggest that one or more additional factors may be required. Rather than being caused by one circulating cytokine, we hypothesize that idiopathic non-familial NS is a multifactorial disease including intrinsic and environmental factors. The search for complex interrelated mechanisms poses a great challenge, and lessons should be drawn from the recent discoveries in the field of familial NS.

As discussed in Chapter 1, mutations in the gene NPHS2 encoding the podocyte specific protein podocin are causative of autosomal recessive steroid-resistant NS. Recent studies suggest that even in some NPSH2 patients, circulating factors play a role in addition to the pathogenic mutation [28]. In a subgroup of NPSH2 patients, proteinuria has been reported to recur shortly after renal transplantation in a manner that would exclude the involvement of antibodies [29-31]. In some patients, the disease is partly responsive to treatment with immunosuppressive drugs, which may point to the involvement of the immune system even in this form of NS [32], although it can not be excluded that immunosuppressive drugs might also directly target components of the glomerular capillary wall. Thus, in some patients, both circulating permeability
factors and podocyte factors are involved in the development of NS. Antignac proposed that allelic variants or heterozygous mutations in NPHS2 or other podocyte genes may modulate the phenotype of a nephropathy that would arise from the presence of a circulating permeability factor; these podocyte gene variants would act not as causative genes, but as phenotype modifiers [28].

The prevalence of podocin mutations in idiopathic non-familial NS patients is currently under investigation. The first reports show that about one fourth of sporadic FSGS is associated with mutations in the podocin gene, whereas no mutations are found in MCNS patients [31]. Other cases of non-familial FSGS have recently been associated with mutations in the gene encoding CD2AP [33]. The search for additional (podocyte) factors involved in the pathogenesis in idiopathic non-familial NS continues. A gene that may encode a yet unknown factor involved in MCNS may be located at chromosome 2p12-p13.2 as has been recently suggested by linkage studies on three siblings with a familial form of MCNS [34].

The involvement of multiple factors, including some intrinsic to podocytes, in the pathogenesis of idiopathic NS does not rule out the possibility of a simple specific therapy. A multifactorial etiology with involvement of multiple cytokines and chemokines has been established for TH2-associated diseases such as asthma and atopic dermatitis; yet therapeutic strategies aimed at one of the cytokines involved have shown promising results [35-37]. Large prospective multicenter studies that will include genetic linkage analysis are warranted to further unravel which factors are involved in the pathogenesis of idiopathic NS and to develop rational targeted therapies.

References

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CHAPTER 7

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