Immune-mediated podocyte injury and the idiopathic nephrotic syndrome
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Chapter 1

General Introduction

Glomerular filtration and Nephrotic Syndrome

The kidney produces urine by which water, electrolytes and toxic metabolites are excreted, while essential nutrients are retained to the body. Each human kidney is composed of 600,000-800,000 functioning units, nephrons. A nephron consists of a filter, the glomerulus, and an ingenious tubular system in which the glomerular filtrate is further processed and transported. The urine is practically devoid of high molecular weight proteins, which is achieved by specific sieving characteristics of the glomerular filtration barrier and by resorption of proteins in the proximal tubules. Many glomerular diseases result in a pathological increase of glomerular permeability to proteins. If proteinuria exceeds 40 mg/m²/hour, hypoalbuminemia accompanied by edema will ensue. The clinical picture of proteinuria, hypoalbuminemia and edema is referred to as “nephrotic syndrome” (NS).

Idiopathic Nephrotic Syndrome

The term “idiopathic nephrotic syndrome” is often used to describe a heterogeneous group of proteinuric glomerulopathies that occur predominantly in children. Over the last few years it has become recognized that some forms of NS formerly assigned as “idiopathic” NS are caused by mutations in genes that encode structural components of the glomerular filter (as reviewed in [1]). Although mainly familial, sporadic cases of these diseases have been described [2, 3]. Clinically, they are characterized by therapy-resistance and eventual progression to end-stage renal failure.

Non-familial forms of NS are more common. Based on the renal biopsy findings, non-familial idiopathic NS can be grossly subdivided into minimal change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS). As indicated by its name, renal tissue from MCNS patients shows no changes on light microscopy. More explicitly, there are no signs of inflammation, immune complex deposition or fibrosis. FSGS is characterized by collapse of the glomerular capillaries with sclerosis and hyalinosis and the formation of adhesions to the glomerular tuft.

General Introduction

Minimal Change Nephrotic Syndrome (MCNS)

In children, MCNS is the most common form of NS, accounting for 35-80% of the cases of NS, depending on ethnicity [4-9]. In adults, MCNS is the third most common form of NS, next to membranous nephropathy and FSGS, accounting for 15-25% of the cases of unexplained adult NS [10]. MCNS is more common in Hispanics, Asians, Arabs, and Caucasians than in African-Americans [9, 11-14] Depending on race, the reported incidence of MCNS varies from 2-16 per 100,000 children under 16 years [11, 15-17]. Previous studies report higher incidences of MCNS than more recent studies do, probably because the incidence of FSGS has increased over time while the incidence of MCNS may be decreasing [9, 10, 18].

Clinically, MCNS is characterized by highly selective proteinuria, i.e. mainly albuminuria, which generally responds well to treatment with corticosteroids. Approx. 90% of children with MCNS and up to 70% of adult patients will respond completely to a course of corticosteroids [7, 19], yet are prone to relapse. Relapses are most frequent in children. In a follow-up study, 95% of children with biopsy-proven MCNS relapsed during 17 years follow-up [20]. Lower relapse rates have been reported, depending on the therapy regimen [21, 22]. If responsive to steroids, the outcome of the disease is excellent. Long-term studies with a follow-up of up to 20 years have shown that practically none of the children with steroid-sensitive biopsy proven MCNS develop hypertension, loss of renal function or FSGS [20, 23, 24]. In adults, the outcome is similar [25-27]. Therefore MCNS is often referred to as steroid-sensitive NS.

In frequent relapsing patients and in steroid-dependent patients, prolonged or repeated steroid therapy can lead to a variety of serious side effects. In these patients, alternative therapeutic strategies can be used to induce long-lasting remission. These include other drugs that modulate the immune system, such as cyclosporine [28-31] and levamisole [32-35], and alkylating agents, such as cyclophosphamide [36-38] and chlorambucil [39].

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Focal segmental glomerulosclerosis (FSGS)

FSGS is a non-specific pattern of glomerular injury that is frequently encountered in human renal biopsies. In adults, only some cases represent idiopathic FSGS, whereas in children, almost all cases are idiopathic. Clinically, FSGS is characterized by increased urinary protein excretion and decreasing kidney function. In idiopathic FSGS, proteinuria is massive and associated with NS. In non-idiopathic forms, FSGS develops secondary to other processes that affect the glomerulus, such as hyperfiltration and hypertension, or scarring following inflammatory, proliferative, or thrombotic glomerulopathies; usually, these cases present with less extensive proteinuria [18]. The incidence of idiopathic FSGS has progressively increased and FSGS now constitutes the most frequent diagnosis in native adult kidneys, accounting for 35-50% of cases of adult NS, depending on race [10]. In children, FSGS is the second most frequent cause of idiopathic NS, accounting for 20-32% of NS [40]. The diagnosis of idiopathic FSGS is most frequent in African-Americans. It has become clear that a significant proportion of children with steroid-resistant FSGS, both familial and non-familial, have mutations in the gene that encodes for the podocyte protein podocin (see below) [2, 3].

The large heterogeneity of the disease is illustrated by the various responses to treatment with corticosteroids. Idiopathic FSGS, both in children and in adults, may respond to corticosteroids [41, 42], yet many patients show steroid-dependence [43, 44]. Steroid-dependent and steroid-unresponsive patients may benefit from treatment with cyclosporine or cyclophosphamide [43-48]. However, more prolonged therapy than in MCNS is generally required to induce remission. Progression of FSGS lesions leads to renal insufficiency and finally to end-stage renal failure, requiring renal replacement therapy. Upon transplantation, the disease relapses in 20-50% of patients [49, 50].

MCNS and FSGS: one disease?

There has been a long debate as to whether or not MCNS can evolve into FSGS. There have been reports on some cases of NS in children who were initially corticosteroid responsive with presumed minimal change histology, but later progressed to renal failure [51]. Ahmad and Tejani reported that in more than 50% of 49 MCNS patients, the renal disease evolved into FSGS over a 10-yr period of repeated renal biopsies [52]. However, the natural history of many cases of MCNS and FSGS is often hard to define, since a few children undergo initial renal biopsy before corticosteroid treatment is started [20]. It has been suggested that long-standing proteinuria may contribute to the transformation of MCNS to FSGS [53]. Studies on familiar NS would support the hypothesis that MCNS and FSGS are part of the same disease spectrum [54], since patients with the same genetic defect in the gene exhibit a range of pathologies from minimal changes to FSGS [3]. Altogether, it is still unclear whether MCNS and FSGS are etiologically related. Here we will discuss MCNS and FSGS as separate entities.

The glomerular capillary wall

The glomerular filtration barrier consists of three layers: (1) the endothelium lining the inner side of the glomerular capillaries; (2) the glomerular basement membrane (GBM) and (3) the glomerular visceral epithelial cells or podocytes that cover the outer side of the glomerular capillary loops.

The endothelium is highly fenestrated. The fenestrae are not covered by diaphragms, thereby constituting effective pores. The individual pores measure between 70 and 100 nm, which is about one order of magnitude larger than plasma proteins. The endothelial cells are attached to the GBM, which is a continuous meshwork of various extracellular matrix proteins, including type IV collagen, laminin, fibronectin and heparan sulfate proteoglycans such as agrin and perlecan. The GBM is produced by both the endothelium and the podocytes. Podocytes are polarized differentiated cells, which owe their name to their complex shape that appears to be designed to allow ultrafiltration. The cell bodies have numerous primary, secondary and tertiary extensions, the latter called foot processes or peduncles. The foot processes cover the GBM in an interdigitating pattern so that adjacent foot processes are derived from different epithelial cells (Figure 1A and B). On the basal side, the foot processes adhere to the GBM. Laterally, the foot processes are interconnected by slit diaphragms through which the filtration route is arranged (Figures 1C and 3). The apical side of foot processes extends into the urinary space. How the glomerular
Capillary wall actually functions as a filter is still a matter of debate. For years, it has been assumed that the normal glomerular capillary wall discriminates filtration of macromolecules based on their size, charge and conformation [55-59]. In this concept, the size-selective barrier is thought to consist of pores in the GBM-meshwork and of the podocyte slit diaphragms, whereas charge-selectivity is thought to be the result of polyanionic sialoproteins and glycosaminoglycans (GAG) that cover both the endothelium and the podocyte foot processes, and the GAG present in the glomerular basement membrane (GBM). These properties would make the filter largely impermeable to proteins. Filtration of large proteins (more than 150 kDa) would be restricted by their size; passage of small polyanionic plasma proteins (70 to 150 kD), primarily albumin, would be restricted by the anionic charge of the glomerular capillary filter. More recently, others have stated that the glomerular capillary wall is permeable to albumin [60-63]. They suggested that in the normal kidney, the filtered albumin is reabsorbed by the proximal tubules, a process which would become impaired or saturated with a fall of the glomerular filtration rate [63]. However, the existence for such massive tubular albumin retrieval is subjected to discussion [64].

Podocytes and proteinuria

NS is associated with dramatic changes in podocyte architecture, as detected by electron microscopy. These changes consist of loss or effacement of the podocyte foot processes (Figure 2) and suggest a process of transdifferentiation. Podocyte foot process effacement appears a uniform response of the podocyte to damage, since it can be induced by several types of injury. The pathogenic mechanism can involve both intrinsic changes within the glomerular filter and extrinsic damage to the glomerular filter.

As stated above, the genetic basis for several forms of hereditary nephrotic syndrome have been solved recently, and the causative genes indeed all appear to affect a single cell type: the podocyte. The extensive effacement of podocyte foot processes in MCNS biopsies, in the absence of immune deposits, inflammation, fibrosis, or abnormalities of the GBM or endothelium, also suggests a pivotal role for the podocyte in the pathogenesis of MCNS. The nature of the initiating podocyte injury in MCNS is as yet unknown, and could still involve intrinsic and extrinsic mechanisms.

Figure 1. The glomerular capillary wall
Cross sections of the glomerular capillary wall in a normal human renal biopsy as revealed by electron microscopy. Cell bodies of podocytes (P) extend into the urinary space, while their foot processes attach to the GBM. In normal individuals, foot processes are arranged in a regular, interdigitating pattern (B) with interconnecting slit diaphragms (C, small arrows). CL, capillary lumen; E, endothelium. A x11,000; C x108,000.

Figure 2. Podocyte foot process effacement in MCNS
Cross sections of the glomerular capillary wall in a patient with MCNS. The podocyte foot processes are irregularly flattened. CL, capillary lumen; US, urinary space. x11,000.
In the next paragraph we will discuss mechanisms of podocyte injury and proteinuria that have been shown causative in human hereditary nephrotic syndromes and in various experimental animal models. These mechanisms are independent of inflammation or immune complex formation, and may therefore be of relevance for MCNS.

**Mechanisms of podocyte injury and proteinuria**

**Direct toxic damage to podocytes**

Puromycin aminonucleoside and adriamycin are compounds that are directly toxic to podocytes. Injection of these toxins into experimental animals causes podocyte foot process effacement and aseleective proteinuria [65-69]. In humans, administration of pamidronate in the course of active treatment for malignancy has been shown toxic to podocytes, causing proteinuria with collapsing glomerulopathy [70]. Collapsing glomerulopathy is a distinct morphological variant of FSGS that may occur in patients infected with human immunodeficiency virus (HIV), known as HIV-associated nephropathy. In HIV-associated nephropathy intracellular localization of the virus appears directly toxic to the podocyte, by affecting cell cycle control [71]. Similar changes have been observed in experimental models of viral infection of the podocytes [72].

Under stress podocytes produce free oxygen radicals that are directly toxic to the podocytes and also damage the GBM. Damage by oxygen radicals has been implicated in the mechanism underlying podocyte damage in many experimental models, among which puromycin aminonucleoside nephrosis [73], but also in congenital nephrotic syndrome of the Finnish type (see below) [74]. In Mpv17-knock out mice, it is primarily the overproduction and extracellular release of oxygen radical species that causes heavy proteinuria and complete podocyte flattening [75].

**Damage to the slit diaphragm**

The functional filter that is formed by the slit diaphragm is primarily the junction between two adjacent foot processes that are derived from two different podocytes. Several observations suggest that the complex of proteins that comprise the slit diaphragm serve as a signaling nexus. Genetic disruption of slit diaphragm components and intracellular slit diaphragm linker proteins (depicted in Figure 3) have been shown to result in proteinuria and distorted podocyte foot processes both in mice and in humans.

Mutations of the gene encoding nephrin were identified as the pathogenic cause of congenital nephrotic syndrome of the Finnish type (CNF) [76]. CNF is characterized by autosomal recessive transmission, extensive flattening of podocyte foot processes and massive aseleective proteinuria occurring already in utero with the need for bilateral nephrectomy and renal transplantation directly after birth. In mice genetic disruption of the nephrin gene causes a concordant phenotype [77]. Mice that lack the Src family kinase Fyn, which tyrosine phosphorylates nephrin, also exhibit podocyte foot process effacement [78, 79]. Mice deficient for the gene encoding nep1, a nehrin homologue that also localizes at the slit diaphragm, show pathological changes comparable to nephrin knockout mice, with development of nephrotic syndrome at birth [80]. A human homologue of this disease has not yet been identified.

Mutations in the gene encoding the stomatin-like protein podocin, which binds and stabilizes nephrin and nep1, cause cases of autosomal recessive early-onset NS [81]. This disease is characterized by early-onset NS with a poor response to corticosteroids and rapid progression to end-stage renal disease. Concordantly, podocin deficient mice show extensive podocyte foot process effacement and develop proteinuria and mesangial sclerosis [82].

Mice deficient for CD2AP, a protein that links nephrin with cytoskeletal components such as F-actin, develop a severe nephrotic syndrome associated with foot process effacement and die soon after birth [83]. Recently two patients with FSGS were identified that had a mutation predicted to ablare expression of one CD2AP allele [84].

Targeted deletion of the fat1 gene, which encodes another adhesion protein that localizes to the podocyte slit diaphragm, results in a loss of slit diaphragms and extensive effacement of the podocyte processes in mice [85]. The precise function of FAT1 at the slit diaphragm and its interaction with other slit diaphragm components remain to be established.

Not only intrinsic damage to the slit diaphragm, by absence of indispensable components, but also
Extrinsic damage caused by antibodies directed against slit diaphragm components disrupts the slit diaphragm by degeneration of the slit diaphragm meshwork. Injection of antibodies against nephrin and nephr1 causes selective proteinuria in mice [86, 87]. Circulating antibodies against nephrin have been implicated in the recurrence of proteinuria in CNF patients after kidney transplantation [88, 89].

Aberrant regulation by transcription factors

Transcription factors are proteins that regulate the transcription and eventually the protein expression of genes. Since accurate expression of slit diaphragm proteins apparently is crucial for the formation and maintenance of podocyte foot processes and correct glomerular permeability, so
should be the regulation of their transcription. This critical role is highlighted by the finding that mutations in WT1, a zinc-finger transcription factor, underlie the pathogenesis of Denys-Drash syndrome [90] and Frasier syndrome [91]. Nephrotic syndrome with podocyte abnormalities is a component of these syndromes, associated with early onset mesangial sclerosis in Denys-Drash syndrome and with FSGS in Frasier syndrome. Truncation of the WT1 protein in mice by an exonic point mutations induces mesangial sclerosis [92]. Combining WT1-knockout and inducible artificial chromosome transgenic mouse models, it was shown that reduced expression levels of WT1 result in glomerular disease, which depending on the gene dosage varies from crescentic glomerulonephritis to mesangial sclerosis [93]. Known downstream targets of WT1 include podocalyxin [94] and Pax2. The transcription factor Pax2 is downregulated by WT1; on the other hand, Pax2 modulates the expression of WT1. Overexpression of Pax2 in transgenic mice results in a phenotype with resemblance to CNF [95].

The transcription factor Lmx1B regulates expression of CD2AP, podocin and collagen IV in podocytes [96, 97]. Mutations in the gene encoding Lmx1B cause the nail-patella syndrome in humans, which is associated with proteinuria in 40% of the patients [98-100]. Concordantly, homozygous knock-out mice generated by targeted inactivation of the Lmx1B gene fail to develop foot processes [96, 97].

PodL1 is basic-helix-loop-helix transcription factor expressed in mesenchyme and podocytes. Its crucial role in podocyte differentiation and glomerulogenesis was illustrated by PodL1 knockout mice that develop immature glomeruli with podocytes that are growth arrested and fail to form foot processes [101]. Mutations in the transcription factor Kreisler (Krm11/MafB) also cause abnormal podocyte differentiation in mice, associated with proteinuria and podocyte foot process effacement [102].

Disruption of the podocyte cytoskeleton
The podocyte foot processes are equipped with a microfilament-based contractile apparatus that provides stability of cell-cell and cell-matrix contacts. A crucial role for maintaining the infrastructure of the membrane domains and for the stability of the podocytes shape is assigned to the submembranous actin cytoskeleton. Indeed mutations in the gene encoding α-actinin-4, which is an actin-filament crosslinking protein localized to the podocyte foot processes, have been identified as the pathogenic cause of a familial form of FSGS with an autosomal dominant inheritance [103]. Transgenic mice that express α-actinin-4 containing a mutation analogous to a mutation described in a human FSGS family in a podocyte-specific manner, show a corresponding phenotype [104] and mice deficient in α-actinin-4 have severe glomerular disease including podocyte distortion and proteinuria [105]. Actin cytoskeleton reorganization is regulated by various other factors, including the Rho small G protein family members. Mice deficient for RhoGDIsa exhibit severe podocyte damage and massive proteinuria [106].

Damage via apical cell-surface proteins
The apical membrane constitutes the major proportion of the foot process cell surface and extends into the urinary space. Here the podocyte is covered by a negatively charged surface coat that is primarily made up of podocalyxin, a negatively charged membrane sialoglycoprotein that is linked to the actin cytoskeleton. Neutralization of the negative charges by perfusion with polycationic compounds, such as protamine sulfate, causes rapid flattening of foot processes and proteinuria in isolated rat kidneys by a mechanism that involves intracellular signaling [107-109]. Podocalyxin knockout mice show distorted podocytes that lack foot processes and slit diaphragms, which does not allow ultrafiltration [110]. In contrast, genetic disruption of the membrane receptor tyrosine phosphatase GLEPP-1, which is also apically located, causes only subtle effects on the anatomy of podocytes and no proteinuria [111].

Injection of antibodies directed against other epitopes that are preferentially located at the apical membrane of the podocyte, such as podoplanin [112] and aminopeptidase A [113] causes rapid effacement of podocyte foot processes and proteinuria in mice by mechanisms that are not yet fully understood.

Damage to cell adhesion receptors
The podocyte foot process is firmly attached to the GBM via an anchoring system spanning the cell membrane. Podocytes express an α3β1 integrin which mediates podocyte adhesion to collagen,
fibronectin and laminin. Integrins not only mediate adhesion, but also transduction of cellular responses by cell signaling [114]. The crucial role of α3β1 in podocyte adhesion and/or signaling is illustrated by the finding that homozygous α3 mutant mice fail to develop foot processes [115].

Disruption of the GBM

Upstream of the podocytes, the GBM constitutes a meshwork with important sieving characteristics. It is composed of various extracellular matrix proteins, including type IV collagen, laminin and heparan sulfate (HS) proteoglycans. HS proteoglycans are thought to constitute the majority of the polyanionic sites in the GBM [116, 117]. The relevance of HS for permselectivity of the GBM is illustrated by several experimental data, showing that enzymatic digestion of renal HS ex vivo by heparitinase causes an enhanced permeability of the GBM for native ferritin and albumin [118-120]. Also, intravenous injection of antibodies directed against glomerular HS causes acute selective proteinuria in rats [121]. Disruption of the GBM meshwork by inactivation of the S-laminin/laminin β2 chain in knock-out mice results in massive proteinuria due to failure of the glomerular filtration barrier [122], while the GBM appears structurally intact.

Podocyte injury in MCNS

As outlined above, there are several ways that lead to podocyte foot process effacement and proteinuria. This is implicated by the complex interactions of the various components that regulate the maintenance of the podocyte shape and function. As shown, isolated or combined damage to any of the interacting components in the podocyte machinery or in the GBM can set in chain a mechanism that eventually causes proteinuria. The extensive effacement of podocyte foot processes as the hallmark of the disease suggests also a pivotal role for the podocyte in the pathogenesis of MCNS.

A genetic linkage study on 15 families with familial MCNS showed that the disease was not linked to the gene encoding podocin [123]. Since MCNS rarely occurs familial, it appears unlikely that the disease is caused by an isolated mutation in a single gene encoding a crucial podocyte protein. Yet several other studies have aimed at investigating the expression of the above-mentioned podocyte proteins in renal biopsies of MCNS patients, to show their possible involvement in the pathogenesis of the disease. Such studies used semi-quantitative immunohistochemical assays, in situ hybridization and reverse-transcriptase polymerase chain reactions (RT-PCR) and have shown conflicting results. In this respect, the expression of nephrin protein has been shown unaltered [124] but also diminished [125, 126] in MCNS biopsies. The expression of nephrin mRNA was reportedly increased [126], decreased [127] or unaltered [124]. A decreased expression of podocin and podoplanin proteins has been reported in glomeruli from nephrotic patients, including MCNS, whereas the mRNA expression of these components would be increased [126]. The expression of synaptopodin [128] and GLEPP1 [129] by podocytes appeared decreased in MCNS. The expression of α3β1 integrin was reportedly normal [130, 131]. A major drawback of the studies outlined here is that the methods used do not allow reliable quantification, which would also explain the sometimes conflicting findings, and that they are often performed on small numbers of biopsies. Furthermore, since they are merely descriptive, they do not allow distinction between primary events and epiphenomena, and the findings are often not specific for MCNS but also present in other proteinuric diseases. An exception is the study performed by Regel et al., who demonstrated that the expression of the αβ dystroglycan complex at the soles of podocyte foot processes was specifically decreased in MCNS compared to FSGS, as shown by immunohistology and immuno-electron microscopy [132].

Since MCNS is characterized by selective proteinuria, mainly albuminuria, special attention has been paid to the anionic charge of the glomerular capillary wall during the disease. It was found that in biopsies from MCNS patients, the anionic charge of the glomerular capillary filter is diminished [133-136]. Van den Born et al. showed that this is associated with loss of HS moieties in the GBM [137].

Taken together, it is still unclear whether MCNS may be caused primarily by an intrinsic defect at any level in the glomerular filtration barrier. Alternatively, podocyte injury in MCNS may result from extrinsic damage caused by a circulating factor. The possible role of circulating factors has been favored by many studies and will be addressed below.
Circulating permeability factors

For FSGS, the involvement of a circulating permeability factor in its pathogenesis has been suggested by several lines of evidence. Proteinuria frequently recurs in the donor kidney of FSGS patients after receiving a non-FSGS kidney transplant [138-140]. Furthermore, it has been reported that proteinuria in two FSGS kidneys disappeared after transplantation in two recipients [141]. Likewise, in a rat model of FSGS, the spontaneously proteinuric Buffalo/Mna rat model, disease recurs in the donor kidney from healthy control rats transplanted into Buffalo/Mna recipients whereas proteinuria and renal lesions regress when the Buffalo/Mna kidneys are transplanted into normal control rats [142]. These data suggest that the FSGS kidney does not bear the causative defect.

Also, the efficacy of plasmapheresis in the treatment of proteinuria in FSGS patients [143-146] and the transmission of proteinuria from an FSGS mother to her child during gestation [147] point to the presence of a causative factor in plasma. The latter is supported by experimental data, showing the induction of albuminuria in rats upon intravenous administration of plasma from FSGS patients [148, 149]. Also, serum from FSGS was shown to increase the permeability of isolated glomeruli to albumin in an in vitro system [150, 151].

For MCNS, the involvement of a circulating permeability factor in the pathogenesis is less well established, due to the fact that MCNS by definition does not require renal transplantation and therefore clinical transfer studies are not present. In MCNS, the hypothesis of a pathogenic circulating factor is merely based on experimental data, showing induction of albuminuria in rats upon injection of culture supernatants of stimulated peripheral blood mononuclear cells (PBMC) from patients with MCNS [152-154] or of a factor produced by human T cell hybridomas derived from a MCNS patient in relapse [155]. Also, the association of MCNS with lymphoproliferative disease, in particular Hodgkin's disease [156, 157], T cell lymphoma [158] and thymoma [159] and the induction of remission by removal of the tumor suggested that circulating factors might play a causal role in the mechanism of proteinuria.

Several candidate permeability factors have been identified to date; however, most have only been partially characterized biochemically. Some of these factors are not entirely specific for any particular glomerular lesion causing NS. Also, it is still unknown what would be the constituent of the glomerular capillary wall that represents the target of such permeability factors [160]. Yet the data presented above suggest that a putative circulating factor in MCNS would be produced by PBMC or, more specifically, T cells, pointing to a role of the immune system.

The role of the immune system

In 1974, Shalhoub proposed that MCNS was a disorder of lymphocyte function with increased levels of a lymphocyte-derived permeability factor [161]. This hypothesis was based on several clinical observations that suggested the involvement of the immune system in the pathogenesis of idiopathic NS. In 1959, Harwicke et al. first reported on a patient with NS in association with pollen hypersensitivity [162]. Since then, many reports have been published on patients who developed after having experienced allergic reactions to inhaled allergens [163-168], vaccinations [169, 170], food [167, 171-175], and insect stings [176, 177]. Furthermore, the incidence of atopy was reportedly higher in patients with idiopathic NS than in healthy subjects, ranging from 17-40% in MCNS patients versus 10-23% in age-matched control subjects [178-184].

Allergy is associated with an elevated production of IgE by B lymphocytes, and several investigators have reported an elevation of IgE in the serum of NS patients [175, 185, 186]. Studies on the glomerular presence of IgE in NS kidneys are controversial [187, 188]. Later experimental studies specifically focused on the role of T lymphocytes, in search of the putative circulating T cell factor described above. Peripheral blood, PBMC, or subsets of T cells were collected from patients with idiopathic NS, and compared with control patients. Frank et al. showed that CD8-positive T cells of idiopathic NS patients are clonally expanded, which was not observed in healthy controls [189]. Sahali et al. showed high levels of nuclear factor-kB DNA binding activity in T cells from untreated MCNS patients during relapse, compared to the MCNS patients in remission while treated with immunosuppressants.
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[190]. This all points to activation of the T cells in MCNS.

Other workers have focused on the production of cytokines by PBMC or T lymphocytes. Cytokines are small proteins (molecular mass of approximately 8-80 kDa) that function as soluble mediators in an autocrine or paracrine manner. Cytokines are produced by immune cells and non-immune cells, and their targets also include both immune and non-immune cells. Based on their profile of cytokine production, T helper lymphocytes can be divided into Th1 and Th2 cells. Th1 cells typically produce interferon-γ, tumor necrosis factor (TNF)-β and interleukin (IL)-2, which activate cytotoxic and inflammatory reactions. By contrast, Th2 cells produce IL-4, IL-5, IL-9, IL-10 and IL-13, which are associated with the regulation of strong antibody and allergic responses [191, 192]. For instance, in asthma, cytokines IL-10 and IL-13, produced by activated Th2 lymphocytes, directly act on pulmonary fibroblasts and bronchial epithelium and thereby cause an important part of the phenotype [193-196].

Studies on in vitro mitogen-stimulated production of cytokines by PBMC from patients with idiopathic NS demonstrated an increased production of various cytokines, including IL-1 [197], IL-2 [197, 198], IL-4 [198, 199] and TNF-α [200], compared with patients in remission or with healthy controls. Matsumoto et al. reported a decreased production of IL-1 and IL-10 by PBMC from MCNS patients and increased production of IL-12 and IL-18 compared with normal controls [201-204]. To circumvent artifacts induced by stimulation with mitogens, Kimata et al. studied the unstimulated production of cytokines by T lymphocytes of MCNS patients, and found an increased production of IL-13, whereas production of IL-4 was normal [205]. An elevated expression of IL-13 mRNA was shown by Yap et al., using a semi-quantitative RT-PCR technique [206]; this was associated with decreased expression of TNF-α and CD14 in monocytes [207]. It has been shown that the increased expression of IL-13 was not related to known polymorphisms in the IL-13 gene [208] or in the genes encoding the transmembrane receptors for IL-4 and IL-13 [209, 210].

The studies mentioned above all investigated only a set of preselected cytokines. Using a subtractive cDNA library screening technique, Sahali et al. applied an unbiased technique and reported differential expression of transcripts involved in the T cell receptor-mediated complex signaling cascade and a decreased expression of IL-12 receptor β2 mRNA by PBMC in untreated MCNS patients during relapse compared with MCNS patients in remission while treated with immunosuppressants [211].

The studies described here not only revealed the possible involvement of T cells and, more specifically, Type2-mediated immunity in the pathogenesis of idiopathic NS, they also illustrated the difficulties that are encountered when studying samples of patients with idiopathic NS. It is difficult to obtain a homogeneous patient group. At time of presentation, the duration of proteinuria varied and some patients have already started treatment. Treatment with immunosuppressive drugs will affect not only the production of the presumed permeability factor, but also of many other factors that are not involved in the disease. Furthermore, it may be difficult to select a correct control group, which would ideally consist of the same NS patients, when in remission without taking any medicine; this may seem most feasible in the frequent-relapsing patients, yet these patients are often steroid-dependent. Also, in the nephrotic state the composition of circulating blood changes, which itself may activate the immune system.
Aim of the study

The first part of this study was undertaken to establish the role of Type2 cytokines in the pathogenesis of MCNS. In the second part, we focused on mechanisms that may underlie podocyte foot process effacement and proteinuria in MCNS.

If Type2 cytokines were to act as circulating permeability factors, the podocytes may constitute the target of such permeability factors. Chapters 2 and 3 focus on direct effects of Type2 cytokines on podocytes. The expression of cytokine receptors on podocytes was analyzed in vivo and in vitro. In a transwell system the effects of cytokine exposure on podocyte monolayers were studied, with particular focus on receptor signaling, ion transport and secretion of the lysosomal enzyme cathepsin L.

In the studies described in Chapters 2 and 3, we observed that exposure to IL-4 and IL-13 induced basolateral secretion of protons by podocytes, which would favor the activity of lysosomal enzymes at the basolateral podocyte surface. Heparanase is a lysosomal enzyme that degrades heparan sulfate. In Chapter 5, we studied the expression of heparanase by podocytes and by PBMC from MCNS patients. The glomerular expression of heparanase was related to disease and to the presence of heparan sulfate in the GBM.

In Chapter 4 the expression of a wide variety of cytokines by PBMC from MCNS patients was analyzed during relapse and remission. Only MCNS patients that were free of immunosuppressive treatment were included, and were compared to a control group of patients with NS based on endogenous causes within the glomerulus.

Podocyte foot process effacement seems a stereotypic reaction of podocytes to damage and is not specific for MCNS. The mechanism of foot process effacement and its role in proteinuria are still not fully understood. In Chapter 6 we describe the relationship between proteinuria and podocyte foot process effacement in patients with MCNS and in patients with IgA nephropathy associated with proteinuria.

In Chapter 7, the results of this study will be summarized and discussed.

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