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Immunopathogenesis of idiopathic nephrotic syndrome

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Abstract

Idiopathic nephrotic syndrome is the most frequent glomerular disease in children. The mechanisms underlying its pathophysiology have been investigated by genetic, cellular, and molecular approaches. While genetic analyses have provided new insights into disease pathogenesis through the discovery of several podocyte genes mutated in distinct forms of inherited nephrotic syndrome, the molecular bases of minimal change nephrotic syndrome and focal and segmental glomerulosclerosis with relapse remain unclear. The immune system seems to play a critical role in the active phase of this disease, through disturbances involving several cell subsets, mainly T cells. The innate immune system may also contribute to the immune disorders. In this review, we discuss recent insights from the molecular and immunological findings and their significance in the context of the clinical course of the disease.

Key Words : MCNS, FSGS, podocyte, NFκB, c-maf

Introduction

Idiopathic nephrotic syndrome (INS) is a kidney disease defined by massive proteinuria and hypoalbuminemia. INS is recognized as a common chronic illness in childhood. Primary INS includes three histological variants: minimal-change nephrotic syndrome (MCNS), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy. MCNS and FSGS respectively account for 70% and 20 % of INS in children, and each accounts for 25% of INS in adults [1, 2]. The hallmark of MCNS is the absence of inflammatory injury or immune complex deposition in the glomerulus, whereas FSGS is characterized by adhesion of the glomerular tuft to Bowman's capsule. Ultrastructural analysis shows glomerular morphological changes in the form of foot-process effacement: the podocytes seem fused together, assuming a flattened rather than a foot-like morphology. Membranous nephropathy , which is rare in children, is a distinct glomerular disease characterized by a nephrotic syndrome associated with prominent immune complex deposits between the podocytes and the glomerular basement membrane [3]. While the mechanisms underlying the formation and the shedding of immune complexes in membranous nephropathy are best understood through studies on Heymann nephritis, a mechanistic explanation of the glomerular abnormalities in MCNS and FSGS with relapse remains elusive. In this review, we will discuss the results of recent investigations that may contribute to our understanding of the pathophysiology of these glomerular diseases. Membranous nephropathy, which is a distinct entity, deserves separate consideration.

Whereas MCNS seems to be a single entity, FSGS appears as a heterogeneous disease, with both immune and non-immune causes. In the latter, recent genetic approaches have elucidated several molecular aspects of FSGS by identifying several genes that play a critical role in the glomerular filtration barrier [4-7]. These studies have opened a large field of

investigation that is summarized in a recent excellent review [8].

The molecular relationship between MCNS and FSGS with relapse is unknown. Undoubtedly, some patients with steroid-sensitive MCNS became steroid-resistant and progress to FSGS and renal failure. Conversely, nearly 50% of patients with FSGS are steroid-sensitive but relapse. Given the infrequency of kidney biopsies in children with nephrotic syndromes, the relative prevalence of MCNS and FSGS at the time of presentation is unknown. Similar immunological disturbances have been reported in the two diseases [9]. The most critical parameter of prognosis is the response to steroid therapy: steroid-sensitive MCNS and FSGS share the same benign course. Of children with nephrotic syndromes, 80-90%, presumably MCNS, are steroid-responsive. Overall, 60-80% of steroid-responsive nephrotic children will relapse and about 60% of those will have five or more relapses [10]. Around 60% of MCNS children show steroid dependence. Resistance to therapy will occur in 50% of FSGS and 10% of MCNS patients despite various immunosuppressive treatments (cyclosporine, cyclophosphamide, high-dose steroids) [11]. Such patients often progress to renal failure, requiring dialysis and transplantation. Recurrent disease in the transplant accounts in 30-50% of patients with FSGS, often in the first hours or days following engraftment [12]. This percentage is as high as 80% for the second graft if recurrence caused the lost of the first graft [13]. The number of years to achieve end-stage renal failure predicts the risk of recurrence after transplantation: 50% if end-stage renal failure was achieved in less than 3 years and 10-20% if this period was longer. Recurrence of the disease in the graft places medicine in a blind alley with regard to possible treatment.

Given the short time between engraftment and disease recurrence, one may exclude the generation of an immune response against any component of the allogeneic glomerular filtration barrier, but movement of a circulating factor endowed with pathogenic properties across the glomerular structure has been postulated [14]. The absence of inflammatory lesions

and immune complex deposits within the glomeruli, as well as the rapid relapses following renal transplantation, support the extra-renal origin of a circulating factor. Indirect evidence comes from observations showing that the nephrotic syndrome disappears when an MCNS or an FSGS kidney is transplanted into an INS-free patient [15, 16].

This review focuses on major findings about the immunopathogenesis of MCNS (and likely FSGS with relapse). We discuss recent findings regarding signal transduction and T cell responses in the context of MCNS relapse versus remission. Finally, an alternative mechanism for the pathophysiology of the disease is proposed, which may reconcile the perspectives of immunologists and nephrologists.

Cytokine profile in NS

Various alterations in cytokine production during MCNS have been described by a large number of studies, which, unfortunately, are not all in agreement. This variation might result from the different immunogenetic backgrounds of the patients, or from the heterogeneity of studies on stimulated cells in environments that are far from physiological. It might also be due to the diversity of the dosage techniques, which do not always take soluble cytokine receptors into account. Table 1 summarizes the data in the literature concerning the expression levels of different cytokines in MCNS. It can be deduced that TNFα and IL-13 levels often increase during relapse. Interestingly, the receptors for the cytokines IL-4 and IL-13 are expressed by podocytes in various glomerular diseases, raising the possibility that IL-13 might contribute to the pathophysiology of proteinuria [17]. In fact, Van Den Berg et al. have demonstrated that IL-4 and IL-13 increase transcellular ion transport, but they do not affect permeability to macromolecules [17]. Moreover, in many pathological conditions in which IL-13 is increased, such as asthma, psoriasis, and allergic dermatitis, proteinuria does not occur.

Research on permeability factor

In addition to the clinical observations mentioned above, plasma replacement performed in patients with FSGS who escape drug therapy was successful [18, 19]. Moreover, immediate recurrences after transplantation have been successfully treated by plasma exchanges or plasma immunoadsorption techniques [20-22]. An-other line of evidence suggesting secretion of a permeability factor comes from experiments showing that systemic infusion of supernatants of cultured PBMC or T cells of patients with MCNS relapse induces proteinuria in rats [23-27]. Immunochemical analyses of affinity column eluate fractions that induced proteinuria suggested that the molecular weight of the permeability factor is below 150 kDa. Numerous attempts to isolate this factor have been unsuccessful.

T cell signal transduction in MCNS

More that three decades ago, it was suggested that MCNS is a systemic disorder of T cell function and cell-mediated immunity [28]. This hypothesis was supported by several clinical observations such as the rapid occurrence of relapses upon antigen challenge (infections or immunization), hyporesponsiveness of lymphocytes to mitogens, and decreased delayed hypersensitivity [29]. Allergic manifestations such as contact dermatitis, rhinitis, and asthma might be observed, particularly in children, but they are uncommon in adult patients with MCNS. Intriguingly, MCNS is the most common kidney disease associated with primary immunological disorders such as Hodgkin's lymphoma [30], leukemia [31], and thymoma [32]. The sensitivity to steroid and immunosuppressive therapy is the most important clinical argument in favor the immune origin of the MCNS.

Several T cell populations are expanded during the active phase of the disease, such as CD4+ T cells expressing the CD25 antigen (IL-2 receptor α chain) [33] and CD8+ T cells

expressing the memory T cell marker, CD45RO [34]. The CD25 antigen is mainly expressed by two sets of CD4+ T cells: a large population (~50%) that expresses CD25 following activation by an immunogenic stimulus, and a minor population (~10%) that constitutively expresses CD25 and CTLA 4, a negative regulator of CD28 signaling [35]. The expression of the CD25 antigen during relapses suggests an activation of CD4+ T cells and/or a recruitment of CD4+ CD25+ T cells endowed with T-suppressive functions. The possible recruitment of the CD4+ CD25+ T cell subset during relapses, seems to agree with some findings showing decreased T cell-mediated immunity and reduced lymphocyte response to mitogens [29], but these studies deserve to be revisited in light of new knowledge. Of note, the immune dysfunction is not restricted to CD4+ T cells. Studies of genetic polymorphism in the variable region of the TCR ß-chain have revealed a selective recruitment of some Vβ gene families in peripheral CD8+ T cells from nephrotic patients with frequent relapses [36]. These results suggest a clonal expansion of CD8+ T cells in long-lasting, active disease.

We have recently reported a downregulation of the IL-12 receptor ß2 subunit (IL-12R β2) during relapse in MCNS T cells [37]. On the other hand, the IL-12R β1 chain was normally expressed. The downregulation of the IL-12R β2 is compatible with the low IL-12 cytokine level, as previously reported by Stefanovic et al. [38] and the lack of induction of the Th1 transcription factor T bet that we observed during relapse (unpublished data). These observations, as well as several published reports, support the hypothesis of Th2 polarization during MCNS. An increase in the production of IL-13, a Th2 cytokine, has been reported by independent groups [39]. Valanciuté et al. have shown that the short form of the proto-oncogene c-maf is highly induced during MCNS relapse [40]. C-maf was identified as the first Th2-specific transcription factor that binds to the IL-4 proximal promoter and therefore, regulates Il-4 expression [41]. C-maf may be activated by a signaling pathway involving B7h/ICOS [42]. C-maf is transcribed into several mRNA forms: the short form, c-maf-S,

encodes a protein of 373 amino acids, whereas the long form, c-maf-L, generated by alternative splicing, encodes a protein of 403 amino acids [43]. C-maf belongs to the large maf family, the members of which are characterized by an N-terminal proline/serine/threonine-rich acidic trans-activation domain and a C-terminal basic region containing the DNA-binding domain. C-maf binds as a homo or heterodimer to the DNA palindromic maf recognition element (Mare) that consists of an extended AP-1 motif [44]**.** Early identified as the first Th2-specific transcription factor binding to the IL-4 proximal promoter, c-maf is barely expressed in resting T cells and is mainly induced by signals emanating from proximal events involving TCR activation [45]. Surprisingly, the expression level of IL-4 during relapse is low and even downregulated as compared to its remission phase level in the same patients, as well as to the levels in normal subjects. Only some patients who suffered from active allergic diseases, such as bronchial asthma, concomitantly with MCNS, expressed a significant amount of IL4, but these cases are infrequent. Moreover, despite the increased frequency of allergic diseases in the world, the incidence of MCNS is remarkably stable, thus excluding close relationships between these diseases. Although unexpected in the context of c-maf activation, the low expression level of IL-4 during the relapse phase has been reported by several groups (Table 1). We have observed that c-maf expression shuts down in patients who enter remission, even though the IL4 transcript level increases. This discrepancy raises two questions: what is the mechanism that counteracts the IL4 induction by c-maf in MCNS, and what is the target gene of c-maf in this disease? The sudden drop in IL4 level during remission might predict relapse (unpublished observations), and suggests that IL-4 monitoring could be relevant to controlling the course of the disease.

The rapid relapse of MCNS following immunogenic challenge led us to postulate the possible involvement of the innate immune system, in which the nuclear factor-κB (NF-κB) signaling pathway plays a central role [46]. Moreover, most cytokines whose levels are thought to be

increased during relapses and downregulated during remissions are partly or predominantly regulated by the NF-κB proteins. The NF-κB transcription factors play a central role in the triggering and coordination of both innate and adaptive immune responses by regulating the expression of a wide variety of genes, such as those encoding IL-1, IL-6, IL-2, IL-8, IL12, Rantes, TNFα, LTα, and chemokines [47]. The NF-κB family includes five members: RelA (p65), NF-κB1 (p50; p105), NF-κB2 (p52; p110), c-Rel, and RelB [48]. They have a structurally conserved 300-amino-acids N-terminal region, responsible for DNA-binding, dimerization, and association with IκB inhibitory proteins [47]. The p50 and p52 proteins are generated by proteolytic processing of precursor p105 and p110 proteins, respectively [49]. Each member of the NF-κB family can form homodimers as well as heterodimers. Homodimers of p50 lacking a transactivation domain can be detected in resting T cell nuclei. The main activated form is the heterodimer of p65 and either p50 or p52 [49]. In resting T cells, the NF-κB complex is sequestered in the cytoplasm through the masking of the nuclear localization signal (NLS) on NF-κB by the IκB proteins [50]. Upon activation by a large number of inducers including byproducts of microbial, viral infections and proinflammatory cytokines, cytosolic IκBα is phosphorylated by the IκB kinase (IKK) complex. Phosphorylated IκBα is recognized by the β-Trcp receptor, which targets it for polyubiquitination by an ubiquitin ligase belonging to the SCF (Skp-1/Cul/F box) family [46]. Subsequently, the IκBα protein is degraded by the proteasome, thus unmasking the NLS and allowing the NF-κB proteins to translocate the nucleus to regulate the transcription of their target genes, which encode cytokines and chemokines. After activation of NF-κB, IκBα is rapidly resynthesized and sequesters the NF-κB complex in the cytoplasm by masking the NF-κB protein's NLS, switching off its activity [51]. This negative feedback, resulting from transcriptional activation of the IκBα gene by NF-κB, occurs despite continuous activation by

cytokines or protein kinase activators [52]. NF-κB proteins exert a dual role on Th differentiation: cRel regulates Th1 differentiation by T-cell-dependent and APC-dependent mechanisms, whereas the NF-κB p50 plays a crucial role in the expression of Gata-3, the master Th2 transcription factor [53].

We have shown that peripheral mononuclear cells, including T cells, exhibit high NF-κB binding activity involving the p50/p65 complexes during relapses, which returned to basal levels during remissions. The mechanism underlying persistent NF-κB activation during the active phase of the disease is not well understood. It implies, at least, a downregulation of IκBα gene activity through reduced induction of transcription and increased destabilization of IκBα mRNA, as well as increased proteasome-mediated IκBα degradation, as demonstrated by the accumulation of the phosphorylated form of IκBα in the presence of MG132, a proteasome inhibitor [54]. The downregulation of NF-κB activity during remissions is correlated with decreased production of most cytokines, such as TNFα, IL-8 and IL-13 [55].

This NF-κB activation, involving the NF-κB RelA/p50 or RelA dimer complexes, seems to be responsible for the inhibition of IL-4 induction during the active phase of the disease. Indeed, overexpression of c-maf in T cells induces IL-4-promoter-driven luciferase activity. On the other hand, coexpression of c-maf with NF-κB RelA/p50, or with RelA dimers, inhibits c-maf-dependent IL-4 promoter activity. Overexpression of p50 amplifies c-maf–mediated IL4 gene activation. These results suggest that increased RelA, whether as homodimers or as heterodimers with p50, significantly blocks the induction of IL-4 by c-maf, in contrast to NFκB p50 homodimers. Moreover, the ex-vivo inhibition of the proteasome activity, which blocks NFκB activation, strongly increases the IL-4 mRNA level in MCNS T cells from relapsing patients [40]. One mechanism underlying transcriptional repression of the IL-4 gene relies on mutual expelling of NF-κB/c-maf from their respective DNA binding

sites. The contribution of other mechanisms, such as the recruitment of corepressor complexes, is not excluded. Casolaro et al. have reported such transcriptional interference by the NFATp and NF-κB transcription factors on IL-4 gene activation [56]. Taken together, these results indicate that NF-κB activity plays a critical role in the modulation of IL-4 gene activation. In particular, the NF-κB proteins, except p50, are involved in Th1 polarization as T cells from mice overexpressing a protease-resistant IκB (that inhibits NF-κB activation), or from c-Rel-deficient mice, are unable to mount a Th1 response [57]. In contrast, p50 is the only known NF-κB protein that plays a determinant role in Th2 polarization through the induction of Gata3 [58].

The relapse-related downregulation of the IL-12R β2 chain, absence of induction of T-bet, overexpression of c-maf, and increased IL-13 production are consistent with T cell commitment towards a Th2 phenotype in MCNS, through an IL4-independent mechanism. These findings might explain why patients with MCNS often display a defect in delayed-type hypersensitivity response, suggesting an abnormal Th1-dependent cellular immunity [29]. The low production of IL-4 suggests that a non-classical ("truncated") Th2 phenotype occurs in MCNS. In order to understand the molecular basis of this process, we screened cDNA arrays with multiplex RNA probes from T cells overexpressing both c-maf and RelA. We identified five new c-maf target genes of unknown function, and these are currently under investigation.

The signaling link between c-maf and extracellular signals: the ICOS/C-MIP pathways?

Activation of the T cell receptor provides insufficient signals for effective T cell function. A second set of signals mediated by co-receptors is needed to promote T cell proliferation, lymphokine secretion and effector functions. Among the co-receptors, ICOS, a member of the CD28 family, delivers signals upon interaction with its ligand, B7H, which is expressed by variety of cells including B cells, antigen presenting cells, and epithelial cells. In

contrast to CD28, which is constitutively expressed by T cells, ICOS is expressed only on activated T cells [59]. Following ligation to B7H, ICOS binds to PI3K and activates PDK1 and AKT/PKB. Studies of knockout mice have revealed that ICOS-, or B7H-deficient T cells are functionally associated with a selective deficiency of c-maf and a strong reduction of IL-4 and IL-13 production [60]. Consequently, T-dependent immune response is impaired and, in particular, the production of IgE is reduced, while IgM and IgG levels appear normal [61]. However, the number and maturation of B cells are normal. In contrast to these findings in KO mice, ICOS-deficient human T cells do not display any defect in cytokine expression, T helper differentiation, or Ig isotype switching. On the other hand, serum immunoglobulin levels, including IgA, IgM, and IgG, were severely reduced in ICOS-deficient patients, leading to recurrent infections. The number of B lymphocytes is reduced, and the expression of the antigen CD27 is notably lacking, suggesting that B cells fail to mature toward a memory phenotype. Taken together, these data are consistent with a severe defect in B cell maturation in human ICOS deficiency and point out the role of ICOS in T cell help for B cells. The expression of c-maf in ICOS-deficient patients has not yet been reported.

Several observations argue against an alteration in the B7H-ICOS signaling axis in MCNS and FSGS. First, although IgG levels are reduced, the concentration of IgM is increased, in MCNS, whereas IgE levels are normal, or increased, especially in patients with associated allergic disease [62]. Second, the number and the maturation of B cells are normal in MCNS. Third, the expression of ICOS protein in MCNS is similar to that in normal controls (our unpublished data). Moreover, recent findings suggest that ICOS contributes to IL-4 and IL13 regulation, but is not required for Th2 differentiation [63]. These observations led us to postulate that the upregulation of c-maf in MCNS occurs through an ICOS-independent pathway.

Regardless of the initial molecular events allowing c-maf induction in MCNS, T cell-

proximal signaling involves the activation of c-mip (c-maf inducing protein), an 85-90 kDa protein encoded by a gene previously identified in the human brain [64]. The predicted structure of c-mip includes an N-terminal region containing a pleckstrin homology domain (PH), a middle region characterized by the presence of several interacting domains including a 14-3-3 module, a PKC domain, an Erk domain, and an SH3 domain similar to the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K), and a C-terminal region containing a leucine-rich repeat (LRR) domain. Grimbert et al. have isolated a shortened form of c-mip, Tc-mip (for truncated c-maf inducing protein); the shortening was caused by deletion of 30 amino acids from the N-terminal region of the PH domain [65]. It has been shown that Tc-mip is highly expressed in CD4+ T cells during the active phase in MCNS patients, and promotes c-maf signaling pathway [65]. The expression of c-mip/Tcmip is predominant in a few tissues, including the thymus, fetal liver, T cells, and the kidney. Overexpression of Tc-mip in Jurkat T cells was shown to strongly induce c-maf protein, transactivate the IL-4 gene, and downregulate the IFNγ, thereby committing the T cell to a Th2 phenotype. Filamin-A, a member of the actin/spectrin/dystrophin family of actin-binding proteins, is upregulated along with Tc-mip in MCNS patients, and was also shown to interact with both c-mip and Tc-mip [66]. These results suggest that Tc-mip plays a critical role in the c-maf-mediated Th2 signaling pathway.

Does the innate immune system play a role in the pathophysiology of MCNS?

One clinical characteristic, likely specific for MCNS, is the rapid occurrence of relapses following viral or bacterial infection, particularly in children. This observation might be consistent with a possible dysfunction of the innate immune system. The innate immune response occurs within hours after an infectious challenge and relies on recognition of microbial products by specific germ-line encoded receptors, the Toll-like receptors (TLR),

which are expressed by APCs, macrophages, neutrophils, natural killer cells, epithelial cells, and endothelial cells. Engagement of TLR triggers a signaling cascade involving myeloid differentiation factor 88 (MyD88), IL-1 receptor-associated kinase (IRAK), and TNF receptor-associated factor 6 (TRAF6), resulting in the activation of the NF-κB and AP-1 pathways, which then induce the activation of the inflammatory genes, such as TNFα, IL-1, IL-6 and IL-8 [67]. The strong activation of NF-κB signaling in non-T cell populations during MCNS relapse suggests the involvement of innate immune cells in this process [54]. This hypothesis is further supported by data showing the upregulation of many genes that are recruited in the innate immune response, such as Rantes, TRAF6, IL-1, IFNαR, and 1-8D protein [37]. Activation of APC leads to membrane expression of costimulator molecules, including B7.1, B7.2, and B7H, along with microbial peptides bound to MHC antigens. Upon interaction with APC, naïve T cells are activated and elicit an adaptive immune response. TLR signaling controls the adaptive response by at least two mechanisms: it triggers costimulatory signals and it influences T cell polarization. Whereas the Th1 immune response requires TLR signaling, the component of the innate system that drives activated T cells towards Th2 commitment remains unknown.

Can podocytes mimic immune cell signaling in some pathological situations?

In some children with MCNS, relapses occur very rapidly following viral or bacterial infections, suggesting that activation of the innate immune system is associated with podocyte dysfunction leading to proteinuria. The underlying mechanism, assumed but not formally demonstrated, is that the immune system, perhaps through T cell dysfunction, releases a factor that induces pathological changes in podocytes. Recently, P. Mundel's group provided evidence that another mechanism might account for the podocyte dysfunction. They have shown that infusion of LPS, a microbial byproduct, in mice induces the expression of B7.1

antigen on podocytes and triggers disorganization of the podocyte cytoskeleton through the Toll-like receptor 4 (TLR-4) signaling pathway [68]. The ability of LPS to induce proteinuria in SCID mice excludes the involvement of immune cells in proteinuria. Indeed, mouse podocytes constitutively express the LPS receptor, TLR-4, and its coreceptor CD14. Induction of B7.1 in podocytes precedes the development of proteinuria, since B7-deficient mice are resistant to LPS-induced proteinuria. This model raises several questions. First, whereas viral or bacterial infections are common in childhood, why do only some children develop MCNS? Second, if the induction of B7.1 on podocyte is a major phenomenon, are the nephrin and podocin pathways highly susceptible to B7.1 signaling in podocytes? Third, increased epithelial expression of TLRs, such as in lupus nephritis, is usually associated with inflammatory reactions, but these are lacking in MCNS. Although this mechanism has been demonstrated in a murine model, the relevance of this finding to the pathophysiology of human nephrotic syndrome remains to be investigated.

Downregulation of activation markers in MCNS is correlated with remission of nephrotic syndrome

Glucocorticoids, the first line therapy for INS, are among the most potent anti-inflammatory and immunosuppressive agents. They inhibit synthesis of almost all known cytokines and of several cell surface molecules required for immune function, but the mechanism underlying their activity is complex and many aspects of their activities remain unclear. After binding to glucocorticoids, the glucocorticoid receptor dissociates from the "chaperone protein", hsp90, and moves into the nucleus, where it downregulates NF-κB activity, essentially by preventing NF-κB from binding to DNA and/or by increasing IκBα transcription [69]. Most cytokine genes that are upregulated in MCNS do not have a glucocorticoid-responsive element, but their promoter exhibits binding sites for NF-κB, which

may explain the fact that many cytokines are downregulated during remission [70]. The downregulation of NF-κB and c-maf activation correlates with the remission of MCNS [54]. Cyclosporine, largely used in patients who exhibit frequent relapses, represses the activation of the NFκB and CD28 pathways [71]. The NF-κB pathway seems to play an important role in the pathogenesis of MCNS, but the mechanisms involved are far from clear. The failure of anti-TNF therapy to induce remission may suggest that NF-κB activation in MCNS does not result from TNF production (our unpublished data).

Alterations in podocyte structure and function during nephrotic syndrome

Visceral glomerular epithelial cells, also called podocytes, are terminally differentiated cells that line the outer aspect of the glomerular basement membrane. They constitute the final barrier to urinary protein loss by the formation and maintenance of the podocyte foot processes (FP) and the interposed slit diaphragm. The basolateral portion of the FP represents the center of podocyte function and is defined by three membrane domains: the apical membrane domain, the slit diaphragm protein complex, and the sole plate (i.e. the basal membrane domain) [72]. Podocyte FPs contain a contractile and dynamic apparatus composed of actin, myosin II, α-actinin-4, talin, vinculin and synaptopodin [73, 74]. The FP are anchored to the glomerular basement membrane via α3β1 integrin [75] and dystroglycans [76]. Neighboring FPs are connected by the slit diaphragm, which represents the main selectively permeable barrier in the kidney [77]. Our knowledge of slit diaphragm structure comes from genetic studies of familial nephrotic syndromes, which led to identification of several proteins (podocin, nephrin, alpha actinine-4 and Trp6) that are mutated in these inherited forms [4-7]. Of note, no mutation has been reported in MCNS in which the expression of these proteins is unchanged or downregulated [78, 79].

Beside their role in the structural organization of the slit diaphragm, podocin, nephrin

and CD2AP participate in cell-signaling pathways that prevent apoptosis [80]. Nephrin and CD2AP, by binding to a subunit of PI3K, stimulate activation of the serine/threonine kinase, Akt. Hence, afferent signaling coming from the slit diaphragm under physiological conditions seems to prevent podocyte apoptosis. On the other hand, alterations in the structure of the slit diaphragm may lead, via attenuated PI3K/Akt signaling, to the apoptotic cell death of podocytes [81]. It is well established that podocyte number is a critical determinant of glomerulosclerosis and that a decrease in podocyte number leads to progressive renal failure [82].

A possible alternative hypothesis unifying T cell disorder and podocyte dysfunction

The clinical and experimental data compiled so far are difficult to reconcile with a single hypothesis involving either an extra-renal factor alone, or a simple molecular defect in podocyte structure, to account for the pathophysiology of MCNS or FSGS with relapse. Rather, one might consider that functional alterations allowing to nephrotic proteinuria could result from downregulation of transduction pathway(s) playing a key role in slit diaphragm function, such as the nephrin- or podocin-mediated pathway. Immuno-ultrastructural studies have demonstrated that the expression of nephrin is diminished in active MCNS [78]. The failure to restore normal nephrin signaling could be due to an antagonistic pathway that is abnormally upregulated in podocytes in active MCNS or FSGS. This negative crosstalk may involve the NF-kB pathway since significantly increased NF-kB expression has been reported in kidney biopsies of recurrent FSGS [83]. The threshold of NF-κB activation might be reduced, causing the immune response to race. This assumption is supported by clinical observations of frequent relapses occurring in the vicinity of antigen challenge, which is not consistent with a usual secondary immune response. How does this functional antagonism take place in the kidney? We postulate that the circulating factor is somehow linked to the

NF-κB pathway. Of note, the factor is not especially secreted by T cells. We have previously reported a sustained NF-κB activation during MCNS relapse, which might suggests that negative regulation of NF-κB is altered in immune cells. It follows that podocytes and immune cells might share the same molecular defect in NF-kB negative-regulatory pathways. Finally, two mechanisms might account for the pathogenesis of MCNS and FSGS with relapse: (1) an activation of NFκB in immune cells, followed by secretion of a circulating factor that might alter the podocyte signaling, and (2) a lack of efficient negative regulation of NF-κB inducing a downregulation of nephrin signaling in podocyte and consequently proteinuria, as well as production of a plethora of cytokines by immune cells.

Conclusion

Since the early 1970s, INS has been postulated to be a systemic disorder of T cell function. Clinical observations and recent pathophysiological and experimental data introduce an additional level of complexity, that is the potential involvement of the innate immune system. It is conceivable that the innate immune response is triggered by pathogen byproducts that activate Toll receptor-mediated signaling pathways, leading to activation of NF-κB. Alterations in the NF-κB/IκBα regulatory feedback loop during relapses may be correlated to the degree of recruitment of innate immunity. Subsequently, the innate immune response generates a costimulatory signal, which induces an adaptive immune response in which the selective induction of the Tc-mip/c-maf pathway contributes to the commitment of T cells towards a Th2 phenotype (Fig 1).

The central role of the podocyte in the induction of the nephrotic syndrome was recently highlighted by the identification of mutations in several podocyte proteins in families with inherited nephrotic syndrome. Moreover, insights from murine models suggest that podocytes may contribute to the activation of innate immunity by expressing the costimulatory protein

B7-1. How B7 signaling alters podocyte function and disrupts the glomerular barrier remains to be determined. Further studies are required to understand the interplay of immune system disorders and podocyte dysfunction.

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Cytokine	Upregulated	Downregulated	No changes	Références
IL-1	κ		κ κ κ κ	[9], [26], [55], [84], [85], [86]
IL-2	κ κ		κ κ κ κ	[9], [26], [55], [86], [87], [88]
IL-4	κ		κ κ κ κ	[9], [26], [39], [88], [89]
IL-6		κ		[90]
IL-8	κ	κ κ	κ	[9], [87], [88], [91]
IL-10		κ		[92]
IL-12	κ		κ	[38], [93]
IL-13	κ κ			[39], [88]
TNFα	κ κ κ			[26], [55], [86]
IFN□	κ	κ	κ, κ, κ	[9], [86], [87], [88], [93], [94],

Table1 . Summary of data reported in the litterature concerning cytokine production in MCNS.

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