# Pathogenesis of childhood idiopathic nephrotic syndrome: a paradigm shift from T-cells to podocytes

**Kazunari Kaneko, Shoji Tsuji, Takahisa Kimata, Tetsuya Kitao, Sohsaku Yamanouchi, Shogo Kato**
*Osaka, Japan*

***Background:*** **Nephrotic syndrome is the most common cause of kidney disease in children, but its pathogenesis remains unclear. This article reviews the novel aspects of the mechanisms underlying massive proteinuria in minimal-change disease, which is the most common form of childhood nephrotic syndrome.**

***Data sources:*** **This article integrates the findings of a PubMed database search for English language articles published in the past 40 years (from September 1974 to February 2014) using the key words "pathogenesis", "minimal change nephrotic syndrome" or "idiopathic nephrotic syndrome".**

***Results:*** **Unknown humoral factors associated with T-cell dysfunction have been thought to play an important role in the pathogenesis of minimal-change disease. However, recent findings are changing this paradigm, i.e., visceral glomerular epithelial cells (podocytes) may be involved via expression of molecules such as CD80 and angiopoietin-like 4.**

***Conclusions:*** **Recent evidence suggests that minimal-change disease results from interactions between humoral factors and dysfunctional podocytes. In addition to immunosuppressant drugs that target lymphocytes, a biological agent such as an antibody against the abnormal molecule(s) expressed by podocytes may provide novel drug treatment for minimal-change disease.**

***World J Pediatr 2015;11(1):21-28***

***Key words:*** **angiopoietin-like 4;**
**CD80;**
**cytokine;**
**minimal change nephrotic syndrome;**
**podocyte**

**Author Affiliations:** Department of Pediatrics, Kansai Medical University, 2-5-1 Shin-machi, Hirakata, Osaka 573-1010, Japan (Kaneko K, Tsuji S, Kimata T, Kitao T, Yamanouchi S, Kato S)

**Corresponding Author:** Kazunari Kaneko, MD, Professor and Chair, Department of Pediatrics, Kansai Medical University, 2-5-1 Shin-machi, Hirakata, Osaka 573-1010, Japan (Tel: +81-72-804-0101 ext. 2560; Fax: +81-72-804-2758; Email: kanekok@hirakata.kmu.ac.jp)

doi: 10.1007/s12519-015-0003-9


Review article

## Introduction

Nephrotic syndrome (NS) is characterized by heavy proteinuria (urine protein:creatinine ratio ≥2000 mg/g or ≥300 mg/dL, or 3+ protein on urine dipstick), hypoalbuminemia (≤2.5 g/dL), and edema.[1] Leakage of massive amounts of serum proteins into the urine leads to a hypercoagulable state, a higher rate of infectious disease, and the dysregulation of fluid balance. However, 80%-90% of children with NS achieve complete remission after glucocorticoid (GC) therapy. The annual incidence of NS is estimated to be 2-7 per 100 000 children, with a cumulative prevalence of 16 per 100 000 children.[2,3]

Childhood NS is classified into three groups: idiopathic (INS, 90% of cases), secondary (10%), and congenital (<1%). INS is further classified into the two major histological variants: minimal-change disease (MCD, 85%) and focal segmental glomerulosclerosis (FSGS, 10%). It is still debatable whether MCD and FSGS represent different ends of the same disease spectrum with the same underlying pathophysiological processes, or whether they are two distinct disease entities.[4,5] Secondary NS is defined as NS associated with a systemic disease, such as lupus nephritis, Henoch-Schönlein purpura nephritis, or Alport syndrome. Congenital NS is defined as heavy proteinuria starting before the age of 3 months, and may be associated with congenital infections (such as syphilis, toxoplasmosis, or cytomegalovirus) or with mutations of the genes coding for podocyte proteins (such as *NPHS1*, *NPHS2*, and *WT1*).[6]

MCD, which is almost equivalent to steroid-sensitive INS, accounts for the vast majority of cases of childhood NS, but its pathogenesis remains unknown. In this review, we discuss recent research findings and the paradigm shift regarding the likely pathogenesis of MCD (including steroid-sensitive INS).

## Old paradigm for the pathogenesis of MCD

In 1974, Shalhoub[7] proposed that MCD was a disorder of T-cell function resulting in increased plasma levels of lymphocyte-derived permeability factor. This hypothesis

was based on the absence of immune complexes in glomeruli, the rapid response to steroid therapy, the association of MCD with Hodgkin's disease, and the observation that measles infection often induced remission of MCD. The massive proteinuria and hypoalbuminemia that characterize MCD were thought to result from increased permeability of the glomerular capillary wall due to T-cell activation triggered by stimuli such as viral infection or allergens.[3] The most compelling evidence came from experience with renal allografts: NS disappeared when MCD kidneys were transplanted into patients without NS.[8] The following clinical findings further support the concept that vascular permeability factors produced from activated T-cells play an important role in MCD: in patients with MCD, there is a risk of recurrence of the disease when transplanted;[9] placental transfer of proinflammatory cytokines from a mother to a newborn results in neonatal NS;[10] the potential of apheresis monotherapy to induce and maintain complete remission of MCD suggests that circulating factors play an important role in the pathogenesis of MCD.[11]

## Role of cytokines in the pathogenesis of MCD

Since Koyama et al[12] found that injection of supernatants from T-cell hybridomas from patients with MCD relapse into rats caused immediate proteinuria and glomerular podocyte foot process fusion, researchers have tried to identify the circulating factors released from T-cells that increase the glomerular permeability to serum proteins. Cytokines were considered to be the most likely pathogenic factors. Cytokines are small proteins (molecular weight 8-80 kDa) that function as soluble mediators in an autocrine or paracrine manner, which are produced by both immune and non-immune cells. Patients with MCD relapse were found to have increased levels of various cytokines in the serum or urine including interleukin (IL)-2,[13] soluble IL-2 receptor,[13-16] interferon-gamma,[13,17] IL-4,[17,18] IL-12,[19] IL-18,[20] tumor necrosis factor (TNF)-α,[21] and vascular endothelial growth factor (VEGF).[22] Isolation of peripheral blood mononuclear cells (PBMCs) from patients with MCD relapse and measurement of the *in vitro* mitogen-stimulated production of cytokines in the cultured cell supernatants demonstrated increased production of various cytokines including IL-1,[23] IL-2,[17,24] IL-4,[17,18] IL-10,[24] IL-12,[25] IL-18,[20] and TNF-α.[26] Yap et al[27] also reported increased expression of IL-13 mRNA in patients with MCD relapse. However, few studies have investigated the direct effects of specific cytokines on the development of proteinuria. VEGF is known to increase capillary permeability by stimulating the release of nitric oxide and thereby inducing endothelial cell fenestration, but VEGF infusion does not induce proteinuria in rats.[28,29] Other cytokines that may be involved in the pathogenesis of MCD include IL-13 and TNF-α. IL-13-transfected rats develop nephrotic proteinuria and display an MCD-like phenotype.[30] However, proteinuria does not occur in many pathological conditions that are associated with increased levels of IL-13 such as asthma, psoriasis, and allergic dermatitis. Infusion of TNF-α has been reported to result in increased urinary albumin excretion in rats.[29] Furthermore, Raveh et al[31] reported remission of MCD after treatment with infliximab, a chimeric TNF-α monoclonal antibody, in a 13-year-old boy with MCD that was refractory to the standard treatment protocols. However, there is controversy over the relationship between increased production of TNF-α and capillary permeability,[32] and infliximab therapy carries a risk of inducing proteinuria secondary to membranous nephropathy.[33]

## Role of T-cells in the pathogenesis of MCD

The aberrant T-cells in MCD are thought to be T helper (Th) type 2 cells,[34-37] because MCD is often associated with atopy and allergy,[38-40] which are caused by Th2 immunologic responses. The increased serum immunoglobulin (Ig) E level and preservation of IgG4 observed in MCD are also characteristic of a Th2 response.[41-43] However, some observations do not support this hypothesis.[24,44] Our study of Th cell subsets in children with MCD using 3-color flow cytometry found no significant differences in the proportions of Th cell subsets [such as Th0 (naive T-cells), Th1, or Th2 CD4+ cells] or the Th1/Th2 ratio among patients with relapse of NS, patients with remission of NS, and normal controls.[45] It has therefore not been definitively established that MCD is a Th2-dependent disorder.

There are clinical reports of MCD remission after depletion of B-cells using monoclonal antibodies or the anti-CD20 drug rituximab (RTX).[46-48] These reports suggest that there are not any underlying T-cell disorders in MCD. Furthermore, the increased nitric oxide production by B-cells exclusively found in the relapse phase of MCD further supports the possibility of B-cell involvement.[49] These findings indicate that T-cells may not play a central role in the pathogenesis of MCD.

## Limitations to the hypotheses focusing on lymphocyte-derived permeability factors

As described above, studies of alterations in cytokine production in MCD reported variable results. The differences may have resulted from the different immunogenetic characteristics of the patients, or the heterogeneity of the stimulated cells in non-

physiological environments. Lack of documentation of biopsy findings, inclusion of steroid-treated patients, and differences in methodology among studies makes it difficult to determine the factors associated with glomerular permeability. Furthermore, the complex interactions among cytokines make it very difficult to determine which cytokine is increased first.

## New paradigm for the pathogenesis of MCD

A new paradigm for the pathogenesis of MCD has emerged since the discovery by Kestila et al[50] in 1998 that mutations in the gene *NPHS1*, which encodes the podocyte-expressed immunoglobulin superfamily protein nephrin, cause congenital NS in humans. This landmark study led to a substantial increase in our understanding of glomerular biology and physiology. Additionally, the development of proteinuria in lipopolysaccharide (LPS)-injected severe combined immunodeficient mice, which are devoid of T- and B-cells, suggests that this mouse model of MCD may be independent of T- or B-cells.[51] Based on these findings, visceral glomerular epithelial cells (podocytes) have attracted particular attention as a key player in the pathogenesis of MCD.[52,53]

## Podocyte ultrastructure is the final barrier to urinary protein loss

Podocytes are terminally differentiated cells that line the outer aspect of the glomerular basement membrane (GBM). Podocytes form the final barrier to urinary protein loss by the formation and maintenance of podocyte foot processes (FPs) and the interposed slit diaphragms (SDs) (Fig. 1).[54] The SDs are the main selectively permeable barrier in the kidney.[55] Podocyte FPs contain a contractile and dynamic apparatus consisting of actin, myosin II, α-actinin-4, talin, vinculin, and synaptopodin.[56,57] The FPs are anchored to the GBM via α3/β1-integrin[58] and dystroglycans.[59] Our knowledge of SD structure is based on genetic studies of familial NS, which led to identification of SD proteins such as podocin, nephrin, α-actinin-4, and TRPC6. The genes for these proteins may be mutated in inherited NS.[6] In contrast, no mutation has been found in MCD characterized by selective albuminuria associated with FP effacement, where the expression of these proteins is unchanged or downregulated.[60,61] It is easily understandable that various proteins can leak from the impaired SD between podocytes due to reduced nephrin expression in congenital NS of the Finnish type or podocyte detachment in FSGS.[62] However, it remains unclear how albumin can diffuse across the effaced podocyte FP in MCD. The effaced podocyte FPs extensively cover the glomerular capillary wall and podocyte SD density is decreased by 80% at most, with half of the slits displaying a tight-junction-like structure.[63] These structural observations raise questions regarding the route by which albumin passes through the glomerular wall. Tojo et al[62] proposed a receptor-mediated albumin transport mechanism that transports albumin through the podocyte cell body by endocytosis and exocytosis. This mechanism of receptor-mediated transport through podocytes may explain the selective albuminuria observed in MCD.

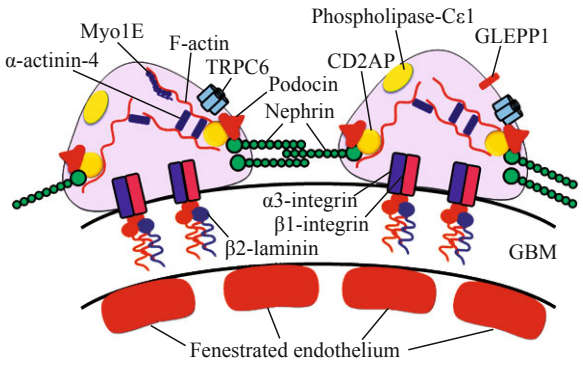


**Fig. 1.** Podocyte proteins that form the foot process ultrastructure and influence glomerular permselectivity.[54] Myo1E: myosin 1E; TRPC6: transient receptor potential cation channel 6; CD2AP: CD2-associated protein; GLEPP1: glomerular epithelial protein 1; GBM: glomerular basement membrane. This figure was reproduced with permission from Springer Science Business Media.

## Hypotheses for the pathogenesis of MCD with a central focus on podocytes

In the last decade, several hypotheses have been proposed that focus on the role of the podocyte in the mechanism underlying the proteinuria in MCD.[53,64-69] Two representative hypotheses are discussed below.

### CD80 expression on podocytes as a key player in the induction of proteinuria

CD80, also known as B7.1, is a T-cell costimulatory molecule that is involved in both activation and termination of the T-cell response. Activation of CD80 on antigen-presenting cells and binding to the CD28 receptor on T-cells has a key role in T-cell activation. In contrast, binding of CD80 to cytotoxic T-lymphocyte-associated (CTLA)-4 terminates the T-cell response.[70] CTLA-4 is expressed on the membrane of the $Foxp3^+$ T-regulatory cell (Treg), and Treg may further inhibit the immune response by release of soluble CTLA-4, IL-10, and transforming growth factor-β (TGF-β). CTLA-4 also suppresses CD80 expression on antigen-presenting cells. Experimental results suggest that direct activation

of podocytes, independent of T-cell involvement, can induce CD80 expression and proteinuria. Injection of LPS into mice increased CD80 expression on podocytes by binding to toll-like receptor (TLR)-4 in association with the development of proteinuria and FP effacement, and LPS also induced CD80 expression in cultured podocytes, with actin reorganization and shape change.[51] CD80 expression can also be induced by T-cell cytokines such as IL-13,[27] and by polyinosinic-polycytidylic acid.[71] Polyinosinic-polycytidylic acid stimulates TLR3 and is structurally similar to the double-stranded RNA found in some viruses, which may be related to the observation that MCD relapse is frequently preceded by an upper respiratory tract infection.[72] Furthermore, MCD is associated with pronounced expression of CD80 on podocytes, and increased urinary excretion of CD80.[66,67] Shimada et al[53] proposed the "two-hit" podocyte immune disorder underlying MCD. Briefly, the "first hit" is induction of podocyte expression of CD80 in response to a circulating factor (such as a cytokine, allergen, or microbial product). The increased CD80 expression on podocytes results in shape change and proteinuria, although the underlying mechanism is unclear.

The proteinuria induced by LPS injection in mice is only transient,[51] and it is hypothesized that CD80 expression in humans is also usually transient because of autoregulatory mechanisms mediated by the T cells and/or podocytes. The "second hit" is dysfunction of this autoregulatory mechanism, resulting in persistent CD80 expression and proteinuria. CD80 expression is inhibited by both CTLA-4 and IL-10[70,73] resulting in resolution of proteinuria. If dysfunctional Tregs in MCD patients cannot turn off podocyte CD80 expression by secretion of soluble CTLA-4, IL-10, and TGF-β, proteinuria may persist. However, this mechanism has not been confirmed in humans. Consistent with this hypothesis, the urinary soluble CD80/CTLA-4 ratio was reported to be >100-fold higher in patients with MCD relapse than in patients with remission,[66] and impaired function of Tregs was observed in MCD patients.[74,75] Evidence that abatacept (CTLA-4-immunoglobulin fusion protein) may inhibit the pathogenesis of rheumatoid arthritis at several levels via selective modulation of CD80/CD86 co-stimulatory molecules expressed by a variety of activated cell types also provides direct and indirect support for this hypothesis.[76]

It is currently unknown why children with MCD may have defective Treg or podocyte autoregulatory function, but this may be related to delayed or ineffective maturation of the T-cell response, possibly because of genetic or environmental factors. Further research is required to determine the mechanism by which CD80 signaling alters podocyte function and disrupts the glomerular barrier.

**Podocyte expressed angiopoietin-like 4 (Angptl4) as a key player in the induction of proteinuria**

Recent studies[52,65,77] found that qualitative and quantitative changes in the expression of Angptl4 in podocytes can induce most of the characteristic features of MCD, including dyslipidemia. Angptl4 is a glycoprotein that shares some structural and functional similarities with angiopoietins, and is expressed in many tissues. Angptl4 inhibits endothelium-bound lipoprotein lipase activity,[78] resulting in increased plasma triglyceride levels. Clement et al[65] reported that glomerular expression of Angptl4 is highly upregulated in the serum and podocytes in experimental models of MCD and in the human disease. Podocyte-specific transgenic overexpression of Angptl4 (NPHS2-Angptl4) in rats induced nephrotic-range proteinuria (over 500-fold increase in albuminuria), loss of GBM charge, and FP effacement. Angptl4 secreted from podocytes in some forms of NS was also shown to lack normal sialylation. Based on these findings, it has been proposed that podocytes secrete a hyposialylated form of Angptl4 in MCD,[52,65,77] whereas a sialylated form of Angptl4 is secreted by extrarenal organs (mostly skeletal muscle, the heart, and adipose tissue) in response to an elevated plasma ratio of free fatty acids to albumin when proteinuria reaches the nephrotic range.

High serum levels of sialylated Angptl4 were found in MCD as well as other glomerular diseases, and these circulating pools of Angptl4 may reduce proteinuria by interacting with glomerular endothelial β5-integrin.[77] Although the precise mechanism underlying the proteinuria caused by podocyte-specific overexpression of Angptl4 is still unclear, the high isoelectric point,[79] positively charged form of Angptl4 may play a key role.[79] Analysis of glomerular protein extracts from the rat model of MCD induced by puromycin aminonucleoside shows that Angptl4 is overproduced in two distinct forms: a positively charged form that migrates at a high pI (8-8.5) and a neutral form that migrates at or just less than a pI of 7. Both high-pI and neutral Angptl4 secreted by podocytes bind to the GBM to alter protein-protein interactions. Progressive accumulation and clustering of Angptl4 in the GBM likely activates signals at the podocyte-GBM interface and induces foot-process effacement, resulting in proteinuria.[52] The relationship between Angptl4 and CD80 has not yet been determined.[52] In a mouse model of MCD, injection with LPS (an activator of TLR4) increased both the expression of Angptl4 in adipose tissue[80] and CD80 on podocytes.[51] It is therefore possible that some pathogenic stimuli such as LPS activate common pathways to induce expression of Angptl4 and CD80 on podocytes.

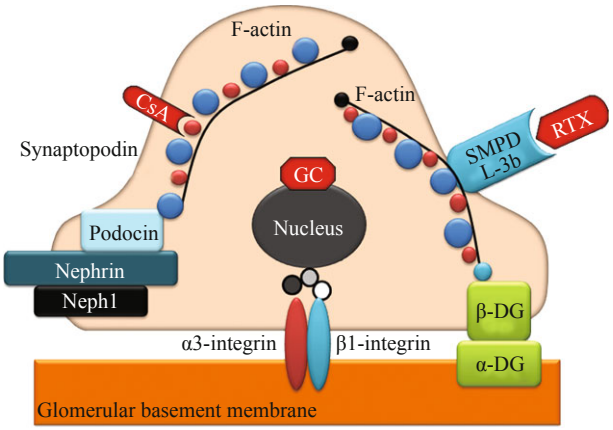


**Fig. 2.** Podocyte as a target for therapies in minimal-change disease. GC inhibit the nuclear factor-kappa B transcription factor signaling in the podocyte. The calcineurin-dependent dephosphorylation of synaptopodin, which in turn leads to destabilization of the podocyte actin cytoskeleton. CsA inhibits this reaction resulting in its stabilization. RTX binds to the CD20 molecule and the podocyte protein SMPDL-3b, which mediates stabilization of the actin cytoskeleton. GC: glucocorticosteroids; CsA: cyclosporine A; RTX: rituximab; SMPDL-3b: sphingomyelin phosphodiesterase acid-like 3b; DG: dystroglycans.

## Podocytes as a novel therapeutic target

Because MCD was historically considered to be a disease of T-cell dysfunction, it was thought that the immunosuppressant drugs used for the treatment of MCD such as GCs, cyclophosphamide, azathioprine, chlorambucil, mycophenolate mofetil, levamisole, cyclosporine A (CsA), tacrolimus, and RTX acted by correcting lymphocyte dysfunction, especially of T-cells. However, it has become clear over the past decade that some of these drugs have direct effects on podocytes, and novel putative modes of action of GCs, CsA, and RTX have been proposed (Fig. 2).[81,82]

Even though 80%-90% of children with MCD achieve complete remission after treatment with GCs, the mechanism by which GCs induce remission is still unknown. As GC receptor expression is ubiquitous, any cell type can theoretically be affected by these drugs. Identification of the podocyte as the key player in MCD resulted in investigation of the effects of GCs on podocytes that may explain their efficacy in MCD. An initial study[83] found that dexamethasone had potent effects on human podocyte structure and function. GCs inhibit the intracellular signaling of nuclear factor κB (NF-κB) transcription factor, which is known to be important in the podocyte.[84] We also confirmed that podocyte NF-κB has a role in the development of proteinuria in a mouse model of MCD induced by puromycin aminonucleoside. Pretreatment with dehydroxymethylepoxyquinomicin (DHMEQ), which potently inhibits the DNA-binding activity of NF-κB, reduced the proteinuria and reversed the serum abnormalities. Electron microscopic analyses indicated that DHMEQ can inhibit podocyte FP effacement by blocking the translocation of podocyte NF-κB from the cytoplasm to the nucleus.[85]

The calcineurin inhibitor CsA is widely used in the treatment of MCD, especially when there is an insufficient response to GCs. Although it was thought that the efficacy of CsA in MCD was due to the inhibition of intracellular signaling in the activated T cells, Faul et al[86] challenged this by demonstrating its action on podocytes. They reported that CsA acts on podocytes by the calcineurin-dependent dephosphorylation of synaptopodin, which in turn leads to destabilization of the podocyte actin cytoskeleton. The net effect is that CsA can stabilize the actin cytoskeleton in podocytes and thereby reduce proteinuria directly. Using sera collected from patients with FSGS recurrence, Fornoni et al[87] recently demonstrated that RTX, a monoclonal antibody directed against CD20 expressed on B-cells, also recognizes CD20 and binds to sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) on podocytes. They confirmed that RTX prevented proteinuria in nephrotic patients by preserving SMPDL-3b expressed on podocytes and by preventing disruption of the actin cytoskeleton of the podocytes.

The recent findings discussed above suggest that the molecules expressed by podocytes in MCD are therapeutic targets for GCs, CsA, and RTX. Novel therapeutic agents directed against these molecules may support the stabilization and reconstruction of podocytes in MCD. For example, inhibition of CD80 expression on podocytes could be of therapeutic interest in MCD, FSGS, and glomerular diseases. Yu et al[88] recently reported treatment of five patients with biopsy-proven CD80 positive podocytes (four with FSGS recurrence and one with primary FSGS) with abatacept, which is a CTLA-4 agonist and CD80 inhibitor, resulting in sustained remission of NS in all cases. Considering both *in vitro* and *in vivo* findings, they suggested that CD80 interferes with the binding of talin to β-integrin, thereby preventing β-integrin activation.[88] However, other findings may not support this suggestion.[89,90]

## Limitations to the hypotheses focusing on podocyte-related molecules

There are several limitations to the hypotheses focusing on podocytes. First, a variety of pathogenic mechanisms contribute to the development of MCD, and there is a growing body of evidence suggesting that pathogenic mechanisms in other cells are also involved, such as alterations in the NF-κB/inhibitory κB regulatory feedback loop of PBMCs,[91] increased

vascular permeability resulting from increased hemopexin production by the liver,[92,93] and increased production of oxygen radicals or nitric oxides by leukocytes.[49,94] It has been suggested that c-maf inducing protein (c-mip) increases in the podocytes in MCD, and that c-mip interferes with podocyte signaling by preventing the interaction of nephrin with the tyrosine kinase Fyn.[69] Second, there is still debate over whether MCD and FSGS represent different ends of the same disease spectrum with the same underlying pathophysiological processes, or whether they are two distinct disease entities.[4,5] There is no clear distinction between steroid-sensitive INS[61] and steroid-resistant INS (FSGS), and findings from studies of each of these conditions are extrapolated to the other condition. Third, importance of the role of CD80 in MCD has been proposed mainly by one group,[53,66-68,71,95,96] and has not been confirmed by other researchers. Finally, the finding that most patients with genetic FSGS did not benefit from CsA[97] suggests that CsA may not have a stabilizing effect. The proposed mechanism of action of RTX on podocytes may also need to be reconsidered in light of the recent suggestion that phagocytes and other inflammatory cells not only remove anti-CD20-opsonized B cells, but also remove autoreactive T-cells that interact with the autoantigen-presenting B-cells.[98]

## Conclusions

Historically, MCD was commonly thought to be caused by T-cell dysfunction. However, recent evidence suggests that lymphocytes and podocytes are both involved in the pathogenesis of this condition, and that MCD results from interactions between humoral factors and dysfunctional podocytes. A biological agent such as an antibody against one or more of the molecules expressed by podocytes could provide a novel drug for the treatment of MCD, in addition to immunosuppressant drugs that target lymphocytes.

## Acknowledgements

The study from our institution cited in the text was partly supported by the Mami Mizutani Foundation.

**Funding:** There was no financial support to prepare the manuscript.

**Ethical approval:** This article does not include any ethical matters to be approved.

**Competing interest:** None declared.

**Contributors:** All authors contributed to the conception and design of the article and the collection of articles to review. Kaneko K performed the literature review, wrote the manuscript, and contributed to revisions of the manuscript for intellectual content. Kaneko K and Kitao T drew the figures. All authors approved the final version of the article.

## References

1 KDIGO. Chapter 3: Steroid-sensitive nephrotic syndrome in children. Kidney Int Suppl (2011) 2012;2:163-171.
2 Eddy AA, Symons JM. Nephrotic syndrome in childhood. Lancet 2003;362:629-639.
3 Kaneko K. Pathogenesis in childhood idiopathic nephrotic syndrome: an update of patchwork. Curr Pediatr Rev 2009;5:56-64.
4 Neuhaus TJ, Fay J, Dillon MJ, Trompeter RS, Barratt TM. Alternative treatment to corticosteroids in steroid sensitive idiopathic nephrotic syndrome. Arch Dis Child 1994;71:522-526.
5 Ahmad H, Tejani A. Predictive value of repeat renal biopsies in children with nephrotic syndrome. Nephron 2000;84:342-346.
6 Jalanko H. Congenital nephrotic syndrome. Pediatr Nephrol 2009;24:2121-2128.
7 Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T-cell function. Lancet 1974;2:556-560.
8 Ali AA, Wilson E, Moorhead JF, Amlot P, Abdulla A, Fernando ON, et al. Minimal-change glomerular nephritis. Normal kidneys in an abnormal environment? Transplantation 1994;58:849-852.
9 Mauer SM, Hellerstein S, Cohn RA, Sibley RK, Vernier RL. Recurrence of steroid-responsive nephrotic syndrome after renal transplantation. J Pediatr 1979;95:261-264.
10 Assadi F. Neonatal nephrotic syndrome associated with placental transmission of proinflammatory cytokines. Pediatr Nephrol 2011;26:469-471.
11 Kobayashi T, Ando Y, Umino T, Miyata Y, Muto S, Hironaka M, et al. Complete remission of minimal-change nephrotic syndrome induced by apheresis monotherapy. Clin Nephrol 2006;65:423-426.
12 Koyama A, Fujisaki M, Kobayashi M, Igarashi M, Narita M. A glomerular permeability factor produced by human T cell hybridomas. Kidney Int 1991;40:453-460.
13 Daniel V, Trautmann Y, Konrad M, Nayir A, Schärer K. T-lymphocyte populations, cytokines and other growth factors in serum and urine of children with idiopathic nephrotic syndrome. Clin Nephrol 1997;47:289-297.
14 Mandreoli M, Beltrandi E, Casadei-Maldini M, Mancini R, Zucchelli A, Zucchelli P. Lymphocyte release of soluble IL-2 receptors in patients with minimal change nephropathy. Clin Nephrol 1992;37:177-182.
15 Hulton SA, Shah V, Byrne MR, Morgan G, Barratt TM, Dillon MJ. Lymphocyte subpopulations, interleukin-2 and interleukin-2 receptor expression in childhood nephrotic syndrome. Pediatr Nephrol 1994;8:135-139.
16 Kemper MJ, Meyer-Jark T, Lilova M, Muller-Wiefel DE. Combined T- and B-cell activation in childhood steroid-sensitive nephrotic syndrome. Clin Nephrol 2003;60:242-247.
17 Neuhaus TJ, Wadhwa M, Callard R, Barratt TM. Increased IL-2, IL-4 and interferon-gamma (IFN-gamma) in steroid-sensitive nephrotic syndrome. Clin Exp Immunol 1995;100:475-479.
18 Cho BS, Yoon SR, Jang JY, Pyun KH, Lee CE. Up-regulation of interleukin-4 and CD23/FcepsilonRII in minimal change nephrotic syndrome. Pediatr Nephrol 1999;13:199-204.
19 Lin CY, Chien JW. Increased interleukin-12 release from peripheral blood mononuclear cells in nephrotic phase of minimal change nephrotic syndrome. Acta Paediatr Taiwan 2004;45:77-80.
20 Matsumoto K, Kanmatsuse K. Elevated interleukin-18 levels in the urine of nephrotic patients. Nephron 2001;88:334-339.
21 Suranyi MG, Guasch A, Hall BM, Myers BD. Elevated levels of tumor necrosis factor-alpha in the nephrotic syndrome in humans. Am J Kidney Dis 1993;21:251-259.

22 Matsumoto K, Kanmatsuse K. Elevated vascular endothelial growth factor levels in the urine of patients with minimal-change nephrotic syndrome. Clin Nephrol 2001;55:269-274.
23 Saxena S, Mittal A, Andal A. Pattern of interleukins in minimal-change nephrotic syndrome of childhood. Nephron 1993;65:56-61.
24 Stachowski J, Barth C, Michalkiewicz J, Krynicki T, Jarmolinski T, Runowski D, et al. Th1/Th2 balance and CD45-positive T cell subsets in primary nephrotic syndrome. Pediatr Nephrol 2000;14:779-785.
25 Matsumoto K, Kanmatsuse K. Increased IL-12 release by monocytes in nephrotic patients. Clin Exp Immunol 1999;117:361-367.
26 Bustos C, Gonzalez E, Muley R, Alonso JL, Egido J. Increase of tumour necrosis factor alpha synthesis and gene expression in peripheral blood mononuclear cells of children with idiopathic nephrotic syndrome. Eur J Clin Invest 1994;24:799-805.
27 Yap HK, Cheung W, Murugasu B, Sim SK, Seah CC, Jordan SC. Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increased IL-13 mRNA expression in relapse. J Am Soc Nephrol 1999;10:529-537.
28 Webb NJ, Watson CJ, Roberts IS, Bottomley MJ, Jones CA, Lewis MA, et al. Circulating vascular endothelial growth factor is not increased during relapses of steroid-sensitive nephrotic syndrome. Kidney Int 1999;55:1063-1071.
29 Laflam PF, Garin EH. Effect of tumor necrosis factor alpha and vascular permeability growth factor on albuminuria in rats. Pediatr Nephrol 2006;21:177-181.
30 Lai KW, Wei CL, Tan LK, Tan PH, Chiang GS, Lee CG, et al. Overexpression of interleukin-13 induces minimal-change-like nephropathy in rats. J Am Soc Nephrol 2007;18:1476-1485.
31 Raveh D, Shemesh O, Ashkenazi YJ, Winkler R, Barak V. Tumor necrosis factor-alpha blocking agent as a treatment for nephrotic syndrome. Pediatr Nephrol 2004;19:1281-1284.
32 Cho MH, Lee HS, Choe BH, Kwon SH, Chung KY, Koo JH, et al. Interleukin-8 and tumor necrosis factor-alpha are increased in minimal change disease but do not alter albumin permeability. Am J Nephrol 2003;23:260-266.
33 Chin G, Luxton G, Harvey JM. Infliximab and nephrotic syndrome. Nephrol Dial Transplant 2005;20:2824-2826.
34 Davin JC, Rutjes NW. Nephrotic syndrome in children: from bench to treatment. Int J Nephrol 2011;2011:372304.
35 Ikeuchi Y, Kobayashi Y, Arakawa H, Suzuki M, Tamra K, Morikawa A. Polymorphisms in interleukin-4-related genes in patients with minimal change nephrotic syndrome. Pediatr Nephrol 2009;24:489-495.
36 Kanai T, Shiraishi H, Yamagata T, Ito T, Odaka J, Saito T, et al. Th2 cells predominate in idiopathic steroid-sensitive nephrotic syndrome. Clin Exp Nephrol 2010;14:578-583.
37 van den Berg JG, Weening JJ. Role of the immune system in the pathogenesis of idiopathic nephrotic syndrome. Clin Sci (Lond) 2004;107:125-136.
38 Hardwicke J, Soothill JF, Squire JR, Holti G. Nephrotic syndrome with pollen hypersensitivity. Lancet 1959;1:500-502.
39 Wittig HJ, Goldman AS. Nephrotic syndrome associated with inhaled allergens. Lancet 1970;1:542-543.
40 Meadow SR, Sarsfield JK, Scott DG, Rajah SM. Steroid-responsive nephrotic syndrome and allergy: immunological studies. Arch Dis Child 1981;56:517-524.
41 Yokoyama H, Kida H, Tani Y, Abe T, Tomosugi N, Koshino Y, et al. Immunodynamics of minimal change nephrotic syndrome in adults T and B lymphocyte subsets and serum immunoglobulin levels. Clin Exp Immunol 1985;61:601-607.
42 Warshaw BL, Check IJ. IgG subclasses in children with nephrotic syndrome. Am J Clin Pathol 1989;92:68-72.
43 Kimata H, Fujimoto M, Furusho K. Involvement of interleukin (IL)-13, but not IL-4, in spontaneous IgE and IgG4 production in nephrotic syndrome. Eur J Immunol 1995;25:1497-1501.
44 Araya CE, Wasserfall CH, Brusko TM, Mu W, Segal MS, Johnson RJ, et al. A case of unfulfilled expectations. Cytokines in idiopathic minimal lesion nephrotic syndrome. Pediatr Nephrol 2006;21:603-610.
45 Kaneko K, Tuchiya K, Fujinaga S, Kawamura R, Ohtomo Y, Shimizu T, et al. Th1/Th2 balance in childhood idiopathic nephrotic syndrome. Clin Nephrol 2002;58:393-397.
46 Bagga A, Sinha A, Moudgil A. Rituximab in patients with the steroid-resistant nephrotic syndrome. N Engl J Med 2007;356:2751-2752.
47 Dotsch J, Muller-Wiefel DE, Kemper MJ. Rituximab: is replacement of cyclophosphamide and calcineurin inhibitors in steroid-dependent nephrotic syndrome possible? Pediatr Nephrol 2008;23:3-7.
48 Kimata T, Hasui M, Kino J, Kitao T, Yamanouchi S, Tsuji S, et al. Novel use of rituximab for steroid-dependent nephrotic syndrome in children. Am J Nephrol 2013;38:483-488.
49 Iharada A, Kaneko K, Tsuji S, Hasui M, Kanda S, Nishiyama T. Increased nitric oxide production by T- and B-cells in idiopathic nephrotic syndrome. Pediatr Nephrol 2009;24:1033-1038.
50 Kestila M, Lenkkeri U, Männikkö M, Lamerdin J, McCready P, Putaala H, et al. Positionally cloned gene for a novel glomerular protein--nephrin--is mutated in congenital nephrotic syndrome. Mol Cell 1998;1:575-582.
51 Reiser J, von Gersdorff G, Loos M, Oh J, Asanuma K, Giardino L, et al. Induction of B7-1 in podocytes is associated with nephrotic syndrome. J Clin Invest 2004;113:1390-1397.
52 Chugh SS, Clement LC, Mace C. New insights into human minimal change disease: lessons from animal models. Am J Kidney Dis 2012;59:284-292.
53 Shimada M, Araya C, Rivard C, Ishimoto T, Johnson RJ, Garin EH. Minimal change disease: a "two-hit" podocyte immune disorder? Pediatr Nephrol 2011;26:645-649.
54 Simic I, Tabatabaeifar M, Schaefer F. Animal models of nephrotic syndrome. Pediatr Nephrol 2013;28:2079-2088.
55 Tryggvason K, Wartiovaara J. Molecular basis of glomerular permselectivity. Curr Opin Nephrol Hypertens 2001;10:543-549.
56 Drenckhahn D, Franke RP. Ultrastructural organization of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat, and man. Lab Invest 1988;59:673-682.
57 Mundel P, Gilbert P, Kriz W. Podocytes in glomerulus of rat kidney express a characteristic 44 KD protein. J Histochem Cytochem 1991;39:1047-1056.
58 Adler S. Characterization of glomerular epithelial cell matrix receptors. Am J Pathol 1992;141:571-578.
59 Regele HM, Fillipovic E, Langer B, Poczewki H, Kraxberger I, Bittner RE, et al. Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. J Am Soc Nephrol 2000;11:403-412.
60 Wernerson A, Duner F, Pettersson E, Widholm SM, Berg U, Ruotsalainen V, et al. Altered ultrastructural distribution of nephrin in minimal change nephrotic syndrome. Nephrol Dial Transplant 2003;18:70-76.
61 Hingorani SR, Finn LS, Kowalewska J, McDonald RA, Eddy AA. Expression of nephrin in acquired forms of nephrotic syndrome in childhood. Pediatr Nephrol 2004;19:300-305.
62 Tojo A, Kinugasa S. Mechanisms of glomerular albumin filtration and tubular reabsorption. Int J Nephrol 2012;2012:481520.
63 Lahdenkari AT, Lounatmaa K, Patrakka J, Holmberg C, Wartiovaara J, Kestila M, et al. Podocytes are firmly attached to glomerular basement membrane in kidneys with heavy

proteinuria. J Am Soc Nephrol 2004;15:2611-2618.
64 Chugh SS, Mace C, Clement LC, Del Nogal Avila M, Marshall CB. Angiopoietin-like 4 based therapeutics for proteinuria and kidney disease. Front Pharmacol 2014;5:23.
65 Clement LC, Avila-Casado C, Mace C, Soria E, Bakker WW, Kersten S, et al. Podocyte-secreted angiopoietin-like-4 mediates proteinuria in glucocorticoid-sensitive nephrotic syndrome. Nat Med 2011;17:117-122.
66 Garin EH, Diaz LN, Mu W, Wasserfall C, Araya C, Segal M, et al. Urinary CD80 excretion increases in idiopathic minimal-change disease. J Am Soc Nephrol 2009;20:260-266.
67 Garin EH, Mu W, Arthur JM, Rivard CJ, Araya CE, Shimada M, et al. Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis. Kidney Int 2010;78:296-302.
68 Ishimoto T, Cara-Fuentes G, Wang H, Shimada M, Wasserfall CH, Winter WE, et al. Serum from minimal change patients in relapse increases CD80 expression in cultured podocytes. Pediatr Nephrol 2013;28:1803-1812.
69 Zhang SY, Kamal M, Dahan K, Pawlak A, Ory V, Desvaux D, et al. c-mip impairs podocyte proximal signaling and induces heavy proteinuria. Sci Signal 2010;3:ra39.
70 Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322:271-275.
71 Shimada M, Ishimoto T, Lee PY, Lanaspa MA, Rivard CJ, Roncal-Jimenez CA, et al. Toll-like receptor 3 ligands induce CD80 expression in human podocytes via an NF-kappaB-dependent pathway. Nephrol Dial Transplant 2012;27:81-89.
72 Alwadhi RK, Mathew JL, Rath B. Clinical profile of children with nephrotic syndrome not on glucorticoid therapy, but presenting with infection. J Paediatr Child Health 2004;40:28-32.
73 Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. Immunol Rev 2008;226:205-218.
74 Araya C, Diaz L, Wasserfall C, Atkinson M, Mu W, Johnson R, et al. T regulatory cell function in idiopathic minimal lesion nephrotic syndrome. Pediatr Nephrol 2009;24:1691-1698.
75 Liu LL, Qin Y, Cai JF, Wang HY, Tao JL, Li H, et al. Th17/Treg imbalance in adult patients with minimal change nephrotic syndrome. Clin Immunol 2011;139:314-320.
76 Cutolo M, Nadler SG. Advances in CTLA-4-Ig-mediated modulation of inflammatory cell and immune response activation in rheumatoid arthritis. Autoimmun Rev 2013;12:758-767.
77 Clement LC, Mace C, Avila-Casado C, Joles JA, Kersten S, Chugh SS. Circulating angiopoietin-like 4 links proteinuria with hypertriglyceridemia in nephrotic syndrome. Nat Med 2014;20:37-46.
78 Yoshida K, Shimizugawa T, Ono M, Furukawa H. Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. J Lipid Res 2002;43:1770-1772.
79 Spink C, Stege G, Tenbrock K, Harendza S. The CTLA-4 +49GG genotype is associated with susceptibility for nephrotic kidney diseases. Nephrol Dial Transplant 2013;28:2800-2805.
80 Lu B, Moser A, Shigenaga JK, Grunfeld C, Feingold KR. The acute phase response stimulates the expression of angiopoietin like protein 4. Biochem Biophys Res Commun 2010;391:1737-1741.
81 Schonenberger E, Ehrich JH, Haller H, Schiffer M. The podocyte as a direct target of immunosuppressive agents. Nephrol Dial Transplant 2011;26:18-24.
82 Mathieson PW. The podocyte as a target for therapies--new and old. Nat Rev Nephrol 2012;8:52-56.
83 Xing CY, Saleem MA, Coward RJ, Ni L, Witherden IR, Mathieson PW. Direct effects of dexamethasone on human podocytes. Kidney Int 2006;70:1038-1045.
84 Hussain S, Romio L, Saleem M, Mathieson P, Serrano M, Moscat J, et al. Nephrin deficiency activates NF-kappaB and promotes glomerular injury. J Am Soc Nephrol 2009;20:1733-1743.
85 Shimo T, Adachi Y, Yamanouchi S, Tsuji S, Kimata T, Umezawa K, et al. A novel nuclear factor kappaB inhibitor, dehydroxymethylepoxyquinomicin, ameliorates puromycin aminonucleoside-induced nephrosis in mice. Am J Nephrol 2013;37:302-309.
86 Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. Nat Med 2008;14:931-938.
87 Fornoni A, Sageshima J, Wei C, Merscher-Gomez S, Aguillon-Prada R, Jauregui AN, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. Sci Transl Med 2011;3:85ra46.
88 Yu CC, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, et al. Abatacept in B7-1-positive proteinuric kidney disease. N Engl J Med 2013;369:2416-2423.
89 Alachkar N, Carter-Monroe N, Reiser J. Abatacept in B7-1-positive proteinuric kidney disease. N Engl J Med 2014;370:1263-1264.
90 Benigni A, Gagliardini E, Remuzzi G. Abatacept in B7-1-positive proteinuric kidney disease. N Engl J Med 2014;370:1261-1263.
91 Sahali D, Pawlak A, Le Gouvello S, Lang P, Valanciute A, Remy P, et al. Transcriptional and post-transcriptional alterations of IkappaBalpha in active minimal-change nephrotic syndrome. J Am Soc Nephrol 2001;12:1648-1658.
92 Lennon R, Singh A, Welsh GI, Coward RJ, Satchell S, Ni L, et al. Hemopexin induces nephrin-dependent reorganization of the actin cytoskeleton in podocytes. J Am Soc Nephrol 2008;19:2140-2149.
93 McCarthy ET, Sharma M, Savin VJ. Circulating permeability factors in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. Clin J Am Soc Nephrol 2010;5:2115-2121.
94 Bertelli R, Trivelli A, Magnasco A, Cioni M, Bodria M, Carrea A, et al. Failure of regulation results in an amplified oxidation burst by neutrophils in children with primary nephrotic syndrome. Clin Exp Immunol 2010;161:151-158.
95 Cara-Fuentes G, Wei C, Segarra A, Ishimoto T, Rivard C, Johnson RJ, et al. CD80 and suPAR in patients with minimal change disease and focal segmental glomerulosclerosis: diagnostic and pathogenic significance. Pediatr Nephrol 2014;29:1363-1371.
96 Ishimoto T, Shimada M, Gabriela G, Kosugi T, Sato W, Lee PY, et al. Toll-like receptor 3 ligand, polyIC, induces proteinuria and glomerular CD80, and increases urinary CD80 in mice. Nephrol Dial Transplant 2013;28:1439-1446.
97 Büscher AK, Kranz B, Büscher R, Hildebrandt F, Dworniczak B, Pennekamp P, et al. Immunosuppression and renal outcome in congenital and pediatric steroid-resistant nephrotic syndrome. Clin J Am Soc Nephrol 2010;5:2075-2084.
98 Datta SK. Anti-CD20 antibody is an efficient therapeutic tool for the selective removal of autoreactive T cells. Nat Clin Pract Rheumatol 2009;5:80-82.

*Received March 31, 2014*
*Accepted after revision July 11, 2014*