Rituximab in Minimal Change Disease: Mechanisms of Action and Hypotheses for Future Studies

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Abstract
Treatment with rituximab, a monoclonal antibody against the B-lymphocyte surface protein CD20, leads to the depletion of B cells. Recently, rituximab was reported to effectively prevent relapses of glucocorticoid-dependent or frequently relapsing minimal change disease (MCD). MCD is thought to be T-cell mediated; how rituximab controls MCD is not understood. In this review, we summarize key clinical studies demonstrating the efficacy of rituximab in idiopathic nephrotic syndrome, mainly MCD. We then discuss immunological features of this disease and potential mechanisms of action of rituximab in its treatment based on what is known about the therapeutic action of rituximab in other immune-mediated disorders. We believe that studies aimed at understanding the mechanisms of action of rituximab in MCD will provide a novel approach to resolve the elusive immune pathophysiology of MCD.

Keywords
minimal change disease, rituximab, nephrotic syndrome, steroid (glucocorticoid)-dependent nephrotic syndrome, frequently relapsing nephrotic syndrome, B cells, T cells

Received September 23, 2016. Accepted for publication November 23, 2016.
**Implications for Future Research/Policy**

This analysis should stimulate future studies into the rationale use of rituximab in common glomerular disorders and inform treatment guidelines and reimbursement regulations.

**Introduction**

Primary nephrotic syndrome, albeit rare, is the most common chronic glomerular disorder in childhood. The vast majority of children presenting with nephrotic syndrome will have minimal change disease (MCD), a clinical and pathological entity characterized by nephrotic-range proteinuria, hypoalbuminemia, hypercholesterolemia, and absence of glomerular immune deposits or cellular infiltrates in the biopsy. The sole histological abnormality is the disappearance of podocyte foot processes (effacement) that is detected by electron microscopy. MCD can also develop de novo in adulthood. The pathogenesis of MCD is not known, but it is believed to be T-cell mediated.1,3

Pediatric MCD is generally responsive to glucocorticoids. As a kidney biopsy is reserved for patients with a complicated or treatment-recalcitrant course, the term MCD is often used interchangeably with glucocorticoid-sensitive nephrotic syndrome (often referred to as steroid-sensitive nephrotic syndrome or SSNS) or idiopathic nephrotic syndrome (INS). However, up to 50% of children with INS experience frequent relapses or glucocorticoid dependence, known as frequently relapsing (FRNS) or steroid-dependent nephrotic syndrome (SDNS). Moreover, about 10% to 20% of the patients show primary or late glucocorticoid resistance (steroid-resistant nephrotic syndrome [SRNS]).

Long-term glucocorticoid use in FRNS/SDNS patients leads to co-morbidities such as cushingoid habitus, growth retardation, striae and acne, reduced bone mineral density, cataracts, pseudotumor cerebri, hypertension, impaired glucose tolerance, hypercholesterolemia, and increased infection risks. Hence, alternative (glucocorticoid-sparing) medications have been introduced. Commonly used “second-line” drugs are oral alkylating agents (mainly cyclophosphamide), calcineurin inhibitors (CNIs, cyclosporine and tacrolimus), levamisole, and mycophenolate mofetil. However, these medications are not uniformly effective in suppressing relapses of proteinuria and are associated with their own spectrum of adverse effects.

During recent years, rituximab (RTX) has been used successfully as a novel treatment modality in patients with INS/MCD. RTX is a chimeric monoclonal antibody containing murine variable regions and a human IgG1 constant domain against CD20. CD20 is a membrane protein expressed on the surface of B lymphocytes, with the exception of late pro-B and plasma cells, and appears to play a role in intracellular Ca\(^{++}\) influx and activation of B cells, and mediates cellular proliferation and differentiation of B cells. RTX causes the elimination of CD20\(^{+}\) B cells by antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity and direct induction of apoptosis. B-cell suppression lasts a few months, but can vary substantially. How RTX works in MCD is not understood and indications for its administration compared with other second-line agents remain to be defined.

The goal of the current review is to explore the mechanisms, by which RTX works in MCD. We will examine the clinical literature on the use of RTX in MCD/INS in children and adults and review available data on the immune pathogenesis of MCD. Finally, we will synthesize hypotheses on the potential mechanisms of action of RTX in MCD, based on the pathophysiology of MCD and what is known about the therapeutic action of RTX in other immune-mediated diseases. We believe that studies aimed at understanding the mechanisms of action of RTX in MCD will provide a novel approach to study the elusive immune pathophysiology of MCD.

**Clinical Studies of RTX in MCD/INS**

**RTX in Children With SDNS/FRNS**

In addition to small case series or specific case scenarios, we identified 4 randomized, controlled trials (RCTs) from 2 centers examining the efficacy and short- and long-term outcomes of RTX treatment in children with glucocorticoid responsive INS (summary in Table 1). The Rituximab for Childhood-Onset Refractory Nephrotic Syndrome Study Group performed a multicenter, double-blind, randomized, placebo-controlled trial at 9 centers in Japan on 48 children with FRNS and SDNS. The reported median relapse-free period was significantly longer in the RTX group than in the placebo group. The authors concluded that RTX is an effective and safe treatment for childhood-onset FRNS and SDNS. Ravanii et al conducted an open-label RCT comprising 54 children with glucocorticoid-dependent and CNI-dependent INS in Italy. The authors noted that RTX along with reduced doses of prednisone and CNI is non-inferior to standard full-dose prednisone plus CNI treatment in maintaining short-term remission. In another RCT by Ravanii et al, 3-month proteinuria was non-inferior in the RTX plus tapered prednisone group compared with the prednisone-only group and median time to relapse was longer in the RTX group. Kidney biopsies were not required or reported in any of the reported trials.

Ruggenenti et al performed a multicenter, off-on trial of RTX in 10 children and 20 adults with different pathologies of INS. All pediatric INS cases were glucocorticoid dependent. Comparing 1-year follow-up after RTX with the year before RTX treatment, per-patient median number of relapses, prednisone maintenance dose, and the median cumulative dose of glucocorticoids to maintain remission were significantly decreased. Overall, there is strong evidence to support the efficacy of RTX in glucocorticoid responsive MCD/INS in children.
| Study                   | Patient characteristics          | Methodology                                                                 | Results                                                                 |
|------------------------|---------------------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Iijima et al\(^{15}\)  | 48 children with FRNS and SDNS  | Multicenter, placebo-control trial, 4 weekly doses of RTX. All patients received standard GC treatment for the relapse at screening and stopped taking immunosuppressive agents by 169 days after randomization; 1-year follow-up | Longer median relapse-free period with RTX (267 days vs 101 days, hazard ratio: 0.27) |
| Ravani et al\(^{16}\)  | 54 children with GC and CNI-dependent INS | Open-label non-inferiority RCT comparing RTX+ reduced dose of prednisone and CNI with standard full-dose prednisone and CNI within 45 days | Three-month proteinuria 70% lower in RTX group; relapse rates 18.5% in RTX vs 48.1% in standard group (P = .029); higher probability of being drug-free at 3 months with RTX (62.9% vs 37%; P < .001) |
| Ravani et al\(^{17}\)  | 46 children with GC and CNI-dependent INS | RTX followed by tapering and withdrawal of oral agents within 45 days | Six-month probability of remission after the first and subsequent RTX infusions: 48% and 37%. One- and 2-year remission probability: 20% and 10% |
| Ravani et al\(^{18}\)  | 30 children with SDNS           | Open-label, non-inferiority RCT. Single RTX infusion in intervention group, continued prednisone in both groups (15 patients each) for 1 month followed by taper as tolerated in 2 months. At least 1-year follow-up | Three-month proteinuria (primary outcome) was non-inferior in RTX group (42% lower in RTX group, geometric mean ratio: 0.58). All but one child in the control group relapsed within 6 months compared with median time to relapse in the RTX group of 18 months |
| Ruggenenti et al\(^{19}\) | 10 children (SDNS) and 20 adults with INS (19 MCD, 3 mesangial GN, and 8 FSGS) | Off-on trial of RTX, comparing 1-year period after RTX with the year before RTX | Significant decrease in per-patient median number of relapses from 2.5 (IQR: 2-4) to 0.5 (IQR: 0-0.1; P < .001), prednisone maintenance dose from 0.27 mg/kg (IQR: 0.19-0.60) to 0 mg/kg (IQR: 0.02-0.3; P < .001), and the median cumulative dose of GC to maintain remission from 19.5 mg/kg (IQR: 13.0-29.2) to 0 mg/kg (IQR: 0-9.4; P < .001) |
| Takei et al\(^{20}\)  | 25 adults with SDNS             | Prospective trial comparing 1-year period after RTX with the year before RTX | Significant reduction in number of relapses (25 [100%] to 4 [16%], P < .001), as well as the total and the maintenance doses of administered prednisolone (8.2 to 3.3 g, P < .001 and 26.4 mg/day to 1.1 mg/day at 12 months, P < .001) |
| Kronbichler et al\(^{21}\) | 86 adults with FRNS/SDNS (MCD or FSGS) | Meta-analysis of 14 studies | RTX reduces the number of relapses per year from 1.3 (0-9) to 0 (0-2), P < .001; proteinuria from 2.43 (0-15) g/day to 0 (0-4.89) g/day (P < .001), and doses of GC-sparing immunosuppressants |
| Guitard et al\(^{22}\) | 41 adults with MCD | Retrospective multicenter study | Complete/partial remission with cessation or reduction of immunosuppressants in 32 (78%) patients following treatment with RTX. After a mean 39-month follow-up, 18 (56%) relapsed and 17 of these received a second course of RTX and then had a complete (n = 13) or partial (n = 4) remission. 9 patients were still in remission at 14 months (3-36) after B-cell recovery |
| Gulati et al\(^{23}\)  | 33 (mostly children) with SRNS (24 with initial and 9 with late resistance) | Four weekly doses of RTX, with continued (reduced) immunosuppressive therapy | Six months after the infusion, 9 (27%) of the SRNS patients were in complete remission, 7 (21%) had partial remission, and 17 (51%) failed to respond; 50% of the non-responders demonstrated progressive CKD or had reached ESRD 12 months after enrollment |
| Prytula et al\(^{24}\) | 70 children with different pathologies of INS from 25 international centers | Questionnaire-based retrospective study | Response rate to RTX of 82%, 44%, and 60% for SDNS/FRNS, SRNS, and recurrent FSGS post-transplant, respectively. Majority of the patients had received GC and/or CNI during and after RTX |

(continued)
Study Patient characteristics Methodology Results

Ito et al 70 children with different pathologies of INS in Japan Questionnaire-based retrospective study 77% (SDNS/FRNS) and 29% (SRNS) patients successfully discontinued prednisone, the majority of them for the first time since disease onset; but 51% relapsed

Kamei et al 10 children with CNI-resistant SRNS Case series; 1-4 doses of RTX followed by methylprednisolone pulse (30 mg/kg/day for 3 consecutive days), every 2-4 weeks until complete remission 7 achieved complete remission, 1 achieved partial remission, and 2 showed no response: 2 with no response progressed to ESRD, 7 with complete remission preserved normal renal function without proteinuria at the last observation

Sun et al 9 children with SDNS/FRNS and 3 with SRNS (7 MCD, 3 FSGS, 1 with focal proliferative glomerulonephritis, and 1 without renal biopsy) Case series; RTX was administered once or twice weekly Total effective treatment rate of RTX was 91.67%, and for 77.78% of the patients, steroid dosage could be reduced. Comparing the six months before and after RTX infusion, the mean steroid dosage was significantly decreased (P = .014) and the number of relapses was significantly reduced (P < .001). The results were better in MCD patients than in FSGS patients (P = .045). There was no significant difference between FSGS/SDNS and SRNS patients (P = .175)

Bagga et al 5 children with SRNS (3 with initial resistance and 2 with late resistance, including 2 MCD and 3 FSGS) Case series; 4 weekly doses of RTX along with continued therapy with CNI, alternate-day prednisolone, or both After 2- to 8-week follow-up, 4 patients had complete remission, and 1 patient had partial remission. Six months later, 1 patient had a relapse and was treated with prednisolone, which resulted in a partial response. Complete remission was maintained in 3 patients, despite the tapering of doses of GC and CNI. The mean ratio of urinary protein to creatinine decreased from 8.3 to 0.8 (P = .02)

Kari et al 4 children with SRNS (2 FSGS, 1 IgM nephropathy, 1 MCD). All were negative for NPHS2 gene mutation Case series. Single-dose RTX None of the patients achieved sustained remission after a single dose of RTX despite effective B-cell depletion

Magnasco et al 31 children with INS unresponsive to combination of CNI and prednisone Open-label multicenter RCT. Two doses of RTX in intervention group. Both groups continued prednisone and CNI for a month, when they started tapering both medications if proteinuria had decreased to < 1 g/day/ m² RTX plus standard treatment failed to induce remission in those who had been previously unresponsive to a combination of CNI and prednisone despite target drug serum levels achieved and CD20 counts despite target drug serum levels and CD20 counts achieved. Three children in each group, all with late resistance to prednisone and alternative agents, entered remission. No subject with primary treatment resistance entered remission in either arm

Fernandez-Fresneda et al 8 adults with biopsy-proven steroid-resistant FSGS At least 4 doses of RTX with concomitant immunosuppressive therapy Only 2 patients experienced a sustained improvement of proteinuria but no patient achieved complete or partial remission

Note. RTX = rituximab; INS = idiopathic nephrotic syndrome; FRNS = frequently relapsing nephrotic syndrome; SDNS = steroid-dependent nephrotic syndrome; GC = glucocorticoid; CNI = calcineurin inhibitor; RCT = randomized clinical trial; MCD = minimal change disease; GN = glomerulonephritis; FSGS = focal segmental glomerulosclerosis; IQR = interquartile range; SRNS = steroid-resistant nephrotic syndrome; CKD = chronic kidney disease; ESRD = end-stage renal disease.
RTX in Adults With SDNS/FRNS

RTX appears to be beneficial also in adults with FRNS/SDNS MCD. Takei et al performed a prospective trial of 25 adults with SDNS and observed that RTX treatment was associated with a reduction in the number of relapses and the total dose of prednisolone needed. The authors concluded that the efficacy of RTX in the treatment of adult SDNS is similar to that in childhood MCD.20 A meta-analysis by Kronbichler et al of 14 studies encompassing 86 adult patients with FRNS/SDNS due to MCD or focal segmental glomerulosclerosis (FSGS), including the data from Takei et al, showed that RTX effectively reduces the number of relapses and doses of glucocorticoid-sparing immunosuppressants.21 Not included in the cited meta-analysis21 is a retrospective multicenter study by Guittard et al who reported complete or partial remission in 78% of adult MCD patients following treatment with RTX.22 Across 15 studies, response rates to RTX (including partial responses) ranged from 63% to 100%. In the absence of RCTs, evidence of the efficacy of RTX in nephrotic syndrome is weaker in adults than in children; nonetheless it appears safe to conclude that RTX is similarly effective in glucocorticoid responsive adult MCD/INS.

RTX in Patients With SRNS

Early case reports suggested that RTX could be a promising agent also in SRNS. However, in contrast to the demonstrated efficacy of RTX in the treatment of SSNS, results in patients with SRNS were inconsistent. Gulati et al studied a largely pediatric cohort involving 33 patients with SRNS from 2 academic tertiary centers in India and the United States.23 Six months after the infusion of RTX, with continued (reduced) immunosuppressive therapy, 27% of the SRNS patients were in complete remission, 21% had partial remission, and 51% failed to respond. A questionnaire-based retrospective study of 70 patients from 25 international centers reported a response rate to RTX of 82%, 44%, and 60% for SDNS/FRNS, SRNS, and recurrent FSGS post-transplant, respectively.24 In another survey of 70 patients in Japan, 77% (SDNS/FRNS) and 29% (SRNS) patients successfully discontinued prednisone, the majority of them for the first time since disease onset. However, 51% of these patients relapsed.25 Other, smaller case series reported variable response rates of SRNS to RTX.26-28 In a series of 4 children with SRNS, none of the patients achieved sustained remission after a single dose of RTX, despite effective B-cell depletion.29 Throughout these studies, the response rate to RTX was consistently lower in SRNS than in SDNS/FRNS, and in most instances, SRNS patients received additional, concomitant immunosuppressants.

An open-label multicenter RCT of 31 children with INS showed that 2 doses of RTX given in addition to standard treatment failed to induce remission in those who had been previously unresponsive to a combination of CNI and prednisone.30 No subject with primary treatment resistance entered remission in either arm. The authors concluded that RTX should not be considered in children unresponsive to glucocorticoids and CNI, especially in those with primary or early unresponsiveness. Similarly, Fernandez-Fresnedo et al reported that none of 8 adults with SRNS and a histological diagnosis of FSGS achieved complete or partial remission of nephrotic-range proteinuria following 4 doses of RTX with concomitant immunosuppressive therapy.31 Thus, RTX, alone or in combination with other immunosuppressants, cannot be recommended to induce or maintain remission in patients with SRNS.32,33

Variable response rates to RTX reported among patients with SDNS/FRNS and SRNS are likely due to heterogeneity in the pathogenesis of “idiopathic” nephrotic syndromes. Tissue diagnosis is missing in some series, especially in glucocorticoid and CNI-responsive patients. Some authors noted a poor correlation between the biopsy diagnosis and responsiveness to RTX.24 Comprehensive genetic testing is rarely available, potentially leading to the inclusion of patients with undiagnosed podocyte gene mutations associated with a lack of response to immunosuppressive therapy.34 Additional genetic factors may modify the responsiveness to RTX. Examples are polymorphisms in genes encoding FcγR,35-38 interleukin (IL)-6,39,40 transforming growth factor (TGF)-β,41 B-lymphocyte stimulator (BLyS), also known as B-cell activating factor (BAFF),42,43 and type I interferon, as shown in patients with rheumatoid arthritis (RA).44,45 Genetic variants may cause incomplete B-cell depletion or unresponsiveness to treatment despite complete B-cell depletion. Loss of RTX in the urine when administered during active nephrotic syndrome is a consideration.46,47 Early (peak) RTX serum levels 24 hours post-infusion were not influenced by the degree of proteinuria.46 Early (peak) RTX serum levels 24 hours post-infusion were not influenced by the degree of proteinuria.46,47 Practically excluding differences in B-cell elimination efficacy; however, proteinuria will likely impact on the duration (maintenance) of B-cell suppression.

Possible Mechanisms of Action of RTX in MCD

As noted above, there is strong clinical evidence that RTX effectively suppresses relapses of proteinuria in patients with glucocorticoid-sensitive MCD. Clinical observations and earlier laboratory studies suggest that the biological changes in MCD are caused by systemic immunological dysregulation and that T cells are the main effector in the process leading to podocyte foot process effacement and resultant heavy proteinuria.1,3 The therapeutic efficacy of B-cell depleting agent not only challenges this dogma, but also affords an opportunity to approach the pathogenesis of MCD from a new angle. In healthy individuals, B cells not only secrete
antibodies, but they also shape protective T-cell responses by providing antigen and co-stimulatory signals, and also produce cytokines that modulate T-cell differentiation. B cells are effector cells in their own right as well. In MCD, post-RTX relapses often occur with B-cell recovery, likely an indication of a pathogenic B-cell population resurgence. Because autoantibodies are not a feature of MCD, B-cell functions other than antibody production should be explored in the quest of understanding how RTX modulates MCD.

Studies focusing on the mechanisms of action of RTX in MCD are limited to the description of B cells during the recovery phase following B-cell depletion, or postulated direct effects of RTX on podocytes. We will discuss possible mechanisms of RTX’s actions in MCD based on current knowledge of its effects in other T-cell–mediated diseases and of the immune pathophysiology of MCD. In Table 2, we reviewed and summarized studies addressing the mechanisms of action of RTX in recognized autoimmune diseases, such as RA, systemic lupus erythematosus (SLE), idiopathic thrombocytopenic purpura (ITP), multiple sclerosis (MS), and type 1 diabetes mellitus (DM1) focusing on non-antibody–mediated aspects of these diseases.

### RTX-Associated Change in the Absolute or Relative Quantity of T-Cell Subsets and B Cells

T cells are divided into subsets with distinct immunological profiles such as CD4+ T helper (Th) cells, which generally

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**Table 2. Effects of RTX in Selected T-Cell–Mediated Diseases.**

| Disease   | Effect of RTX |
|-----------|---------------|
| RA        | 1. T-cell depletion, mainly of Th CD4+ cells, associated with clinical response. Another study failed to observe T-cell depletion. |
| SLE       | 1. Possible inhibition of T-cell co-stimulation. |
| ITP       | 1. Immediate response to RTX while autoantibodies are still present. |
| MS        | 1. Therapeutic effects without changes in serum or CSF antibody levels. |
| DM1       | 1. Although anti–T-cell therapies (eg, anti-CD3 antibody) are effective in treatment of DM1, RTX was also shown to be helpful in new-onset DM1. |

**Note.** RTX = rituximab; RA = rheumatoid arthritis; CD = Cluster of differentiation; (comment: again, CDs are standard abbreviations and much more known than the cluster of differentiation); RANKL = receptor activator of nuclear factor kappa-B ligand; NK = natural killer; IL = interleukin; IFN = interferon; TGF = transforming growth factor; MMP = matrix metalloproteinase; SLE = systemic lupus erythematosus; BAFF = B-cell activating factor; BR3 = BAFF receptor 3; TNF = tumor necrosis factor; ITP = idiopathic thrombocytopenic purpura; MS = multiple sclerosis; CSF = cerebrospinal fluid; DM1 = type 1 diabetes mellitus.
act as immune effectors, CD8⁺ cytotoxic cells, and regulatory T cells (Treg cells; see below). As shown in Table 2, studies of various autoimmune diseases showed that in addition to B-cell depletion, RTX decreases absolute and/or relative numbers of T cells and T-cell subsets, in particular Th cells. In addition, a small, likely pathogenic subset of T cells has been identified in patients with RA; they express small amounts of CD20 (CD20dim) and are targeted by RTX. These cells are characterized by a shift from Th1 to Th17 subtype, associated with T-cell–mediated inflammation. A fraction of IL-17–producing CD3⁺CD4⁻CD8⁻ Th17 subtype, associated with T-cell–mediated inflammation, is also possible that RTX works by targeting a small subset of Th cells. It is also possible that RTX augments the number of regulatory T cells (Treg cells; see below). As shown in Table 2, RTX may increase the number of Treg cells from children in (untreated) relapse, compared with 26 normal children, whereas the overall number of lymphocytes and total T cells, and CD4⁺ and CD8⁻ T-cell counts were unchanged. Others confirmed increased total and relative peripheral CD19⁺ B-cell counts in treatment-naïve children at the onset of SSNS compared with normal controls and otherwise healthy children with a recent viral infection. Lama et al noted a decreased proportion of CD4⁺ T cells both in children with SSNS in relapse and SRNS, whereas Prasad et al showed that Th1 and Th2 cells were increased at the time of relapse and decreased during glucocorticoid-induced remission, measured at least 4 weeks after the discontinuation of prednisone. Significantly greater numbers of CD4⁺ and CD8⁺ memory T-cell subsets (CD45RO⁺CD4⁺ and CD45RO⁺CD8⁺) have been described in patients with active, untreated INS compared with healthy controls.

According to a model proposed by Lund and Randall, RTX-induced B-cell depletion can interrupt a feed forward loop between autoreactive effector B cells and T cells and abrogate consequent inflammation and tissue injury. Such a mechanism may contribute to the effect of RTX in MCD. It is also possible that RTX works by targeting a small subset of T cells that express CD20. Possible roles of specific T-cell subsets including Treg, Th1, Th2, and Th17 cells in MCD are discussed in the following sections.

**Modulation of Treg Cells and Related Cytokines**

Thymus-derived, naturally occurring Treg cells are defined by the combined detection of CD4⁺, CD25⁺, and the transcription factor, Forkhead box P3 (Foxp3). Treg cells function as immune suppressors through several mechanisms, including the production of inhibitory cytokines, IL-10, and TGF-β. Expression of Foxp3 in mature Treg cells is necessary for their suppressive function, whereas loss of Foxp3 expression leads to the production of cytokines characteristic of other Th cell lineages. Maintaining the division between regulatory and effector T-cell lineages appears to be critical for immune homeostasis; loss of Treg cells is associated with autoimmunity and, possibly, chronic inflammation.

Although data presented by Araya et al suggest that the percentage of Treg cells is similar in healthy controls and pediatric patients with MCD in relapse or in remission, others have reported that the number and percentage of peripheral Treg cells (CD4⁺CD25⁻Foxp3⁺) were significantly decreased during active MCD, compared with patients in remission and healthy controls. In contrast, the peripheral Th1 and Th2 cell abundance was increased during active nephrotic states and decreased during remission, along with the ex vivo expression of interferon (IFN)–γ and IL-4, major cytokines of Th1 and Th2 cells, respectively. During remission, Treg cell numbers rose, with a corresponding increase in IL-10 and TGF-β production ex vivo.

Similar findings were noted in adult MCD patients; the peripheral blood Th17/Treg cell ratio and Th17-related plasma cytokines (IL-17 and IL-23) were increased and Treg cells and Treg-related cytokines (TGF-β1 and IL-10) were decreased during proteinuria and normalized after the induction of remission. Jaiswal et al found lower percentages of Treg cells and Treg/Th1 ratios in glucocorticoid resistant MCD, compared with glucocorticoid responsive MCD in remission and healthy controls.

Shao et al suggested a role for a dynamic equilibrium between Th17 (effector) and Treg cells in patients with various glomerulopathies. Interestingly, in the Buffalo/Mna rat model of FSGS, Treg cells were increased in drug-induced remission, and transfer of Treg cells induced remission. The finding is consistent with the notion that decreased Treg cell numbers or activity may contribute to the development or exacerbation of nephrotic syndrome.

As shown in Table 2, RTX may increase the number of Treg cells and restore their defective regulatory functions in autoimmune diseases such as SLE and ITP. Rocatello et al reported an up to 10-fold increase in Treg cells from baseline in patients with active (nephrotic) membranous nephropathy following RTX-induced B-cell depletion and clinical remission. This effect was inversely correlated to the abundance of active CD8⁺ T cells. How RTX augments the number and/or function of Treg cells remains unclear. Nonetheless, it is possible that RTX increases or restores functions of Treg cells in MCD, thereby preventing inflammatory cytokine-induced podocyte injury.

**Th1/Th2 Balance and Related Cytokines**

Th1 and Th2 are 2 subsets of Th cells, each expressing specific markers and representative cytokines. Several studies have addressed potential disturbances of the physiological Th1 and Th2 cell polarity in MCD. Although their findings suggest that Th1/Th2 imbalance may have a contributory or causative role in the pathogenesis of MCD, the origin and significance of the observed imbalance remain
inconclusive.109 Some authors reported increased plasma levels or in vitro expression of the main Th1 cytokines IFN-γ and tumor necrosis factor (TNF)–α in MCD patients95,96,110; one study found that remission of proteinuria correlated with decreased IFN-γ levels.96 However, others failed to observe an increase in Th1 cytokine levels in MCD patients.111-114

Evidence to support a role of Th2 cells in MCD appears somewhat stronger but is not conclusive either. The major Th2 cytokines are IL-4 and IL-13. Increased IL-13 serum levels have been reported in patients with active, relapsing proteinuria,112,114,115 although decreased IL-4 levels have also been reported during relapse.115 Polymorphisms of IL-13 and IL-4 encoding genes and modification of their signaling pathways have been associated with predisposition to MCD and response to treatment,116-118 providing additional support for the possible role of Th2 cells. Receptors for IL-13 and IL-4 are expressed in cultured podocytes, and both cytokines have been shown in vitro to modulate protein trafficking, proteolysis, intercellular junction, and membrane functions in podocytes.119-122 Lai et al reported significant proteinuria in podocytes from MCD patients during relapse, but not in remission, stimulating CD80 expression in cultured podocytes. However, experimental approaches varied widely among studies; investigators chose limited and variable cytokine sets; biological materials used included serum/plasma, urine, peripheral blood mononuclear cells (PBMCs), or supernatant of PBMCs cultured ex vivo with or without stimulation; cytokines were directly measured or mRNA was quantified. Methodological differences and varying or conflicting results complicate drawing definitive conclusions on the roles of each cytokine. Importantly, observed changes in cytokine expression may be the cause or a consequence of MCD exacerbation.3 Nevertheless, RTX has been shown to reduce the levels of a number of plasma or locally expressed cytokines in humans with RA.96 RTX treatment of streptozotocin diabetic rats decreased staining of the proinflammatory nuclear factor, nuclear factor–κB, in the kidney.134 It is therefore possible that RTX acts by altering the expression of certain cytokine(s) and suppressing subsequent inflammation. Of note, several non-cytokine molecules have been proposed to act as circulating permeability factors in INS, such as hemopexin, CLC-1, and suPAR.135 Although not explored to date, it is conceivable that expression of such molecules may be modifiable by RTX.

Modulation of T-Cell Functions by Reducing Co-stimulation

B cells modulate T-cell responses via co-stimulatory molecules.136,137 Co-stimulation can be induced by the interaction of CD40 on B cells with CD40L (CD154) on T cells, and by the interaction of CD80 (B7-1) or CD86 (B7-2) on B cells and other antigen presenting cells with CD28 on T cells. Blockade of CD40-CD40L interaction by antibody has been protective against renal injury in mouse models of chronic proteinuric renal disease,138,139 membranous nephropathy,140 and lupus nephritis.141

In patients with SLE, RTX therapy was associated with a significant reduction in CD40L mRNA and downregulation of CD40L on Th cells as well as downregulation of CD40 and CD80 on remnant B cells. These changes correlated with decreased T-cell activation and a favorable clinical response.66-68 It is conceivable that RTX blocks T-cell–co-stimulatory pathways in MCD, thereby modulating T-cell function.

Interestingly, CD80 can be expressed by podocytes.142 Its urinary excretion is elevated in patients with relapsing MCD compared with patients in remission, patients with other glomerular diseases, and healthy controls.143-146 Sera from MCD patients in relapse, but not in remission, stimulate CD80 expression in cultured podocytes.147 Increased CD80 expression alters podocyte actin cytoskeleton and slit diaphragm protein organization independent of T and B cells.142 Cytotoxic T-lymphocyte–associated protein-4 (CTLA-4, also known as CD152) binds to and blocks CD80.148,149 Yu et al reported that abatacept (a fusion protein composed of the Fc region of immunoglobulin IgG1 and the extracellular domain of CTLA-4), which is used as an inhibitor of CD80, induced partial or complete remission of proteinuria in
patients with primary FSGS or post-transplant recurrent FSGS. The authors suggested that abatacept acts by inhibiting CD80 on podocytes. Although experimental data are convincing, there remains the possibility that abatacept acts by blocking canonical T-cell co-stimulation. Of note, constitutive expression of CTLA-4 by Treg cells is believed to be essential for the immuno-inhibitory functions of these cells. The CTLA-4/CD80 pathway and Treg cell functions in the treatment of MCD warrants further exploration. Another co-stimulatory receptor, CD40, was also found in glomeruli or podocytes, and its pathological role was suggested in post-transplant recurrence of FSGS, although the evidence was indirect.

**Role of Antigen Presentation by B Cells**

B cells present antigens to T cells. Considering that relapses of proteinuria are often triggered by non-specific viral infections, it is tempting to hypothesize that RTX depletes B cells carrying the memory of the viral antigens and that the lack of viral antigen presentation to T cells leads to a long-term remission. It has been suggested recently that the delayed reconstitution of switched memory B cells after RTX-induced B-cell depletion was protective against relapse of MCD. Although the physiological significance of these findings is yet to be understood, it suggests that memory B cells are important in the pathogenesis of MCD relapse.

**Effect on Cytotoxic Cells and Macrophages**

Some studies suggested that active MCD is associated with an increased number of cytotoxic cells as evidenced by a decreased CD4$^\text{+}$/CD8$^\text{+}$ ratio. However, data on a possible role of CD8$^\text{+}$ T cells in MCD are scarce. Natural killer (NK) cell deficiency was associated with frequently replacing MCD in a patient with Hodgkin’s disease, and RTX was shown to increase active NK cells in patients with RA and SLE, suggesting a possible implication of NK cells in the effect of RTX in MCD. However, another study reported functionally normal NK cells in MCD. RTX was also shown to decrease the number of synovial macrophages (Mφ) and to inhibit TNF-α production by Mφ in patients with RA. Although the data suggest a role for TNF-α in some patients with recurrent FSGS post-transplantation, the role of TNF-α and/or Mφ is unknown for MCD. With currently available evidence, there is no strong signal to implicate cytotoxic cells, NK cells, or Mφ in the mechanisms of action of RTX in MCD, and it appears that the field is understudied.

**Direct, Non-immunological Effects on Podocytes**

Although podocytes do not express CD20, Fornoni et al reported that RTX binds to podocytes by targeting sphingomyelin-phosphodiesterase-acid–like 3b (SMPDL-3b), which regulates acid-sphingomyelinase (ASMase) activity. SMPDL-3b and ASMase are essential for the organization of receptors and signaling molecules in highly specialized cells and are involved in the modulation of actin remodeling in podocytes. In Fornoni’s study, RTX partially prevented the downregulation of SMPDL-3b and ASMase in cultured podocytes exposed to FSGS serum; SMPDL-3b overexpression or treatment with RTX prevented disruption of the actin cytoskeleton and podocyte apoptosis induced by patients’ sera. This effect was diminished when the SMPDL-3b gene was silenced. Yoo et al suggested that SMPDL-3b may be an important modulator of podocyte function and a novel therapeutic target in glomerular diseases. Thus, RTX may improve SMPDL-3b activity in podocytes and contribute to its therapeutic effect in MCD.

**Conclusions and Future Research Direction**

The aim of this review was to synthesize hypotheses pertaining to the mechanisms of action of RTX in the induction of prolonged remission in MCD. Insights into these mechanisms can be exploited to study the pathogenesis of MCD; understanding how RTX works in the treatment of MCD is expected to shed new light on the immune pathogenesis of the disease and will possibly lead the identification of the elusive permeability factor(s).

Although the direct protective actions of RTX on podocytes remains a possibility, overwhelming evidence points the involvement of the immune system, in particular T and B lymphocytes. Although the direct actions of lymphocytes on podocytes are yet to be examined, evidence points to the presence of circulating humoral factors responsible for podocyte injury in MCD, whose identity is yet to be uncovered. We would like to propose a hypothesis that in MCD, RTX targets disease-promoting B-cell subsets that promote dysregulated T-cell responses, which are responsible for the secretion of humoral factors that injure podocytes. Alternative hypotheses would be that RTX targets a small number of inflammatory CD20$^\text{+}$ T cells or effector B cells. Recent advances in high-resolution immunophenotyping technologies allow the robust enumeration, stratification, and functional characterization of immune cell subsets including T-cell and B-cell subsets, which we can use to pin-point the immune derangement in MCD. With the increasing number of MCD patients treated with RTX for relapse control, we now have tools and opportunities to test our hypotheses. Efforts toward such investigations are underway in our laboratory in collaboration with the Canadian Childhood Nephrotic Syndrome Project.

**List of Abbreviations**

ASMase, acid-sphingomyelinase; CNI, calcineurin inhibitor; CTLA-4, cytotoxic T lymphocyte–associated protein-4; DM1, type 1 diabetes mellitus; ITP, idiopathic thrombocytopenic purpura;
FSGS, focal segmental glomerulosclerosis; FRNS, frequently relapsing nephrotic syndrome; IL, interleukin; IFN, interferon; MCD, minimal change disease; MR, macrophages; MS, multiple sclerosis; NK, natural killer cells; NKT, natural killer T cells; RA, rheumatoid arthritis; RCT, randomized controlled trial; RTX, rituximab; SDNS, glucocorticoid (steroid)–dependent nephrotic syndrome; SLE, systemic lupus erythematosus; SMPDL-3b, sphingomyelin-phosphodiesterase-acid–like 3b; SRNS, glucocorticoid (steroid)–resistant nephrotic syndrome; SSNS, glucocorticoid (steroid)–dependent nephrotic syndrome; T11, T helper; TNF, tumor necrosis factor; Treg cells, regulatory T cells.

Author Contributions
NM performed literature review and drafted the manuscript. MB contributed to literature review and writing of the manuscript. TT supervised the entire process of manuscript preparation. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
All authors have consented for article publication.

Availability of Data and Materials
Not applicable.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study is supported by grants from the Canadian Institute of Health Research (MOP-53335, MOP-126180 to TT) and the Kidney Foundation of Canada (to TT).

References
1. Eddy AA, Symons JM. Nephrotic syndrome in childhood. Lancet. 2003;362:629-639.
2. Cunard R, Kelly CJ. T cells and minimal change disease. J Am Soc Nephrol. 2002;13:1409-1411.
3. Mathieson PW. Immune dysregulation in minimal change nephropathy. Nephrol Dial Transplant. 2003;18(suppl 6):vi26-vi29.
4. Samuel S, Bitzan M, Zappitelli M, et al. Canadian Society of Nephrology Commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis: management of nephrotic syndrome in children. Am J Kidney Dis. 2014;63:354-362.
5. Gipson DS, Massengill SF, Yao L, et al. Management of childhood onset nephrotic syndrome. Pediatrics. 2009;124:747-757.
6. Schulman SL, Kaiser BA, Polinsky MS, Srinivasan R, Baluarte HJ. Predicting the response to cytotoxic therapy for childhood nephrotic syndrome: superiority of response to corticosteroid therapy over histopathologic patterns. J Pediatr. 1988;113:996-1001.
7. Moghadam-Kia S, Werth VP. Prevention and treatment of systemic glucocorticoid side effects. Int J Dermatol. 2010;49:239-248.
8. Cybulsky AV, Walsh M, Knoll G, et al. Canadian Society of Nephrology Commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis: management of glomerulonephritis in adults. Am J Kidney Dis. 2014;63:363-377.
9. Browning JL. B cells move to centre stage: novel opportunities for autoimmune disease treatment. Nat Rev Drug Discov. 2006;5:564-576.
10. Kamei K, Ito S, Nozu K, et al. Single dose of rituximab for refractory steroid-dependent nephrotic syndrome in children. Pediatr Nephrol. 2009;24:1321-1328.
11. Sinha A, Bhatia D, Gulati A, et al. Efficacy and safety of rituximab in children with difficult-to-treat nephrotic syndrome. Nephrol Dial Transplant. 2015;30:96-106.
12. Sinha A, Bagg A, Gulati A, Hari P. Short-term efficacy of rituximab versus tacrolimus in steroid-dependent nephrotic syndrome. Pediatr Nephrol. 2012;27:235-241.
13. Sellier-Leclerc AL, Baudouin V, Kwon T, et al. Rituximab in steroid-dependent idiopathic nephrotic syndrome in childhood—follow-up after CD19 recovery. Nephrol Dial Transplant. 2012;27:1083-1089.
14. Tellier S, Brochard K, Garnier A, et al. Long-term outcome of children treated with rituximab for idiopathic nephrotic syndrome. Pediatr Nephrol. 2013;28:911-918.
15. Iijima K, Sako M, Nozu K, et al. Rituximab for childhood-onset, complicated, frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet. 2014;384:1273-1281.
16. Ravani P, Magnasco A, Edefonti A, et al. Short-term effects of rituximab in children with steroid- and calcineurin-dependent nephrotic syndrome: a randomized controlled trial. Clin J Am Soc Nephrol. 2011;6:1308-1315.
17. Ravani P, Ponticelli A, Siciliano C, et al. Rituximab is a safe and effective long-term treatment for children with steroid and calcineurin inhibitor-dependent idiopathic nephrotic syndrome. Kidney Int. 2013;84:1025-1033.
18. Ravani P, Rossi R, Bonanni A, et al. Rituximab in children with steroid-dependent nephrotic syndrome: a multicenter, open-label, noninferiority, randomized controlled trial. J Am Soc Nephrol. 2015;26:2259-2266.
19. Ruggenenti P, Ruggiero B, Cravedi P, et al. Rituximab in steroid-dependent or frequently relapsing idiopathic nephrotic syndrome. J Am Soc Nephrol. 2014;25:850-863.
20. Takei T, Itabashi M, Moriyama T, et al. Effect of single-dose rituximab on steroid-dependent minimal-change nephrotic syndrome. Pediatr Nephrol. 2013;28:911-918.
21. Kronbichler A, Kerschbaum J, Fernandez-Fresnedo G, et al. Rituximab treatment for relapsing minimal change disease and focal segmental glomerulosclerosis: a systematic review. J Am Soc Nephrol. 2014;39:322-330.
22. Guitard J, Hebral H, Fakhouri F, et al. Rituximab for minimal-change nephrotic syndrome in adulthood: predictive factors for response, long-term outcomes and tolerance. Nephrol Dial Transplant. 2014;29:2084-2091.
23. Gulati A, Sinha A, Jordan SC, et al. Efficacy and safety of treatment with rituximab for difficult steroid-resistant and -dependent nephrotic syndrome: multicentric report. *Clin J Am Soc Nephrol*. 2010;5:2207-2212.

24. Prytula A, Ijima K, Kamei K, et al. Rituximab in refractory nephrotic syndrome. *Pediatr Nephrol*. 2010;25:461-468.

25. Ito S, Kamei K, Ogura M, et al. Survey of rituximab treatment for childhood-onset refractory nephrotic syndrome. *Pediatr Nephrol*. 2013;28:257-264.

26. Kamei K, Okada M, Sato M, et al. Rituximab treatment combined with methylprednisolone pulse therapy and immunosuppressants for childhood steroid-resistant nephrotic syndrome. *Pediatr Nephrol*. 2014;29:1181-1187.

27. Sun L, Xu H, Shen Q, et al. Efficacy of rituximab therapy in children with refractory nephrotic syndrome: a prospective observational study in Shanghai. *World J Pediatr*. 2014;10:59-63.

28. Bagga A, Sinha A, Moudgil A. Rituximab in patients with the steroid-resistant nephrotic syndrome. *N Engl J Med*. 2007;356:2751-2752.

29. Kari JA, El-Morshedy SM, El-Desoky S, Alshaya HO, Rahim KA, Edrees BM. Rituximab for refractory cases of childhood nephrotic syndrome. *Pediatr Nephrol*. 2011;26:733-737.

30. Magnasco A, Ravani P, Edefonti A, et al. Rituximab in children with resistant idiopathic nephrotic syndrome. *J Am Soc Nephrol*. 2012;23:1117-1124.

31. Fernandez-Fresneda G, Segarra A, Gonzalez E, et al. Rituximab treatment of adult patients with steroid-resistant focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*. 2009;4:1317-1323.

32. Pradhan N, Furth S. Rituximab in steroid-resistant nephrotic syndrome in children: a (false) glimmer of hope? *J Am Soc Nephrol*. 2012;79:471-475.

33. Ito S, Kamei K, Ogura M, et al. Survey of rituximab treatment for childhood-onset refractory nephrotic syndrome. *Pediatr Nephrol*. 2013;28:257-264.

34. Bagga A, Sinha A, Moudgil A. Rituximab in patients with the steroid-resistant nephrotic syndrome. *N Engl J Med*. 2007;356:2751-2752.

35. Kari JA, El-Morshedy SM, El-Desoky S, Alshaya HO, Rahim KA, Edrees BM. Rituximab for refractory cases of childhood nephrotic syndrome. *Pediatr Nephrol*. 2011;26:733-737.

36. Magnasco A, Ravani P, Edefonti A, et al. Rituximab in children with resistant idiopathic nephrotic syndrome. *J Am Soc Nephrol*. 2012;23:1117-1124.

37. Fernandez-Fresneda G, Segarra A, Gonzalez E, et al. Rituximab treatment of adult patients with steroid-resistant focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*. 2009;4:1317-1323.

38. Pradhan N, Furth S. Rituximab in steroid-resistant nephrotic syndrome in children: a (false) glimmer of hope? *J Am Soc Nephrol*. 2012;79:471-475.

39. Madanchi et al. *Rituximab in resistant idiopathic nephrotic syndrome*. *World J Pediatr*. 2014;29:636-641.

40. Fabris M, Quartuccio L, Lombardi S, et al. The CC homozygosis of the -174G>C IL-6 polymorphism predicts a lower efficacy of rituximab therapy in rheumatoid arthritis. *Autoimmun Rev*. 2012;11:315-320.

41. Daien CI, Fabre S, Rittore C, et al. TGF beta polymorphisms are candidate predictors of the clinical response to rituximab in rheumatoid arthritis. *Joint Bone Spine*. 2012;79:471-475.

42. Ruysen-Witrand A, Rouanet S, Combe B, et al. Association between -871C>T promoter polymorphism in the B-cell activating factor gene and the response to rituximab in rheumatoid arthritis patients. *Rheumatology (Oxford)*. 2013;52:636-641.

43. Fabris M, Quartuccio L, Vital E, et al. The TTTT B lymphocyte stimulator promoter haplotype is associated with good response to rituximab therapy in seropositive rheumatoid arthritis resistant to tumor necrosis factor blockers. *Arthritis Rheum*. 2013;65:88-97.

44. Thurlings RM, Boumans M, Tekstra J, et al. Relationship between the type I interferon signature and the response to rituximab in rheumatoid arthritis patients. *Arthritis Rheum*. 2010;62:3607-3614.

45. Raterman HG, Vosslander S, de Ridder S, et al. The interferon type I signature towards prediction of non-response to rituximab in rheumatoid arthritis patients. *Arthritis Res Ther*. 2012;14:R95.

46. Coussilman CE, Jol-van der Zijde CM, Stevens J, Carnsberg K, Bredius RG, Sukhai RN. Pharmacokinetics of rituximab in a pediatric patient with therapy-resistant nephrotic syndrome. *Pediatr Nephrol*. 2015;30:1367-1370.

47. Guigonis V, Dallocchio A, Baudouin V, et al. Rituximab treatment for severe steroid- or cyclosporine-dependent nephrotic syndrome: a multicentric series of 22 cases. *Pediatr Nephrol*. 2008;23:1269-1279.

48. Lund FE, Randall TD. Effector and regulatory B cells: modulators of CD4+ T cell immunity. *Nat Rev Immunol*. 2010;10:236-247.

49. Colucci M, Carsetti R, Caccioli S, et al. B cell reconstitution and renal outcome in congenital and pediatric steroid-resistant nephrotic syndrome. *Arthritis Rheum*. 2010;62:3607-3614.

50. Fornoni A, Sageshima J, Wei C, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med*. 2011;3:85ra46.

51. Piantoni S, Scarsi M, Tincani A, Airo P. Circulating CD4+ T cell immunity. *Nat Rev Immunol*. 2010;10:236-247.

52. Melet J, Mulleman D, Goupille P, Ribortout B, Watier H, Thibault G. Rituximab-induced T cell depletion in patients with rheumatoid arthritis: association with clinical response. *Arthritis Rheum*. 2013;65:2783-2790.

53. Thurlings RM, Vos K, Wijbrands CA, Zwinderman AH, Gerlag DM, Tak PP. Synovial tissue response to rituximab with rheumatoid arthritis: association with clinical response. *Arthritis Rheum*. 2013;65:2783-2790.

54. Feuchtenberger M, Muller S, Roll P, et al. Frequency of regulatory T cells is not affected by transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Open Rheumatol J*. 2008;2:81-88.

55. Takemura S, Klimiuk PA, Braun A, Goronyz JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. *J Immunol*. 2001;167:4710-4718.
56. Boumans MJ, Thurlings RM, Yeo L, et al. Rituximab abrogates joint destruction in rheumatoid arthritis by inhibiting osteoclastogenesis. *Ann Rheum Dis*. 2012;71:108-113.

57. Toubi E, Kessel A, Soblodin G, et al. Changes in macrophage function after rituximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2007;66:818-820.

58. Willeke P, Becker H, Schluter B, et al. Rituximab effectively reduces macrophage migration inhibitory factor in patients with active rheumatoid arthritis. *Scand J Rheumatol*. 2012;41:242-243.

59. Lurati A, Bertani L, Marrazza M, Re KA, Bompane D, Scarpellini M. NK cell count as predictor of clinical response in patients with rheumatoid arthritis treated with rituximab. *Biologics*. 2012;6:83-87.

60. Lurati A, Marrazza MG, Re KA, Scarpellini M. Relationship between NK cell activation and clinical response in rheumatoid arthritis treated with rituximab. *Int J Biomed Sci*. 2009;5:92-95.

61. Diaz-Torre C, Ortiz de, Juana MA, Geli C, et al. Rituximab-induced interleukin-15 reduction associated with clinical improvement in rheumatoid arthritis. *Immunology*. 2014;142:354-362.

62. Hasan E, Olusi S, Al-Awadhi A, Mokaddem K, Sharma P, George S. Effects of rituximab treatment on the serum concentrations of vitamin D and interleukins 2, 6, 7, and 10 in patients with rheumatoid arthritis. *Biologics*. 2012;6:31-35.

63. Gutierrez-Roelens I, Galant C, Theate I, et al. Rituximab treatment induces the expression of genes involved in healing processes in the rheumatoid arthritis synovium. *Arthritis Rheum*. 2011;63:1246-1254.

64. Klimiuk PA, Domyslawska I, Sierakowski S, Chwiecko J. Regulation of serum matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 following rituximab therapy in patients with rheumatoid arthritis refractory to anti-tumor necrosis factor blockers. *Rheumatol Int*. 2015;35:749-755.

65. Tamimoto Y, Horiuchi T, Tsukamoto H, et al. A dose-escalation study of rituximab for treatment of systemic lupus erythematosus and Evans’ syndrome: immunological analysis of B cells, T cells and cytokines. *Rheumatology (Oxford)*. 2008;47:821-827.

66. Sfikakis PP, Bouliotis VL, Fragiadaki KG, Moutsopoulos HM, Boletis JN, Theofilopoulos AN. Increased expression of the FoxP3 functional marker of regulatory T cells following B cell depletion with rituximab in patients with lupus nephritis. *Clin Immunol*. 2007;123:66-73.

67. Tokunaga M, Fuji K, Saito K, et al. Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab. *Rheumatology (Oxford)*. 2005;44:176-182.

68. Sfikakis PP, Boletis JN, Lionaki S, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum*. 2005;52:501-513.

69. Iwata S, Saito K, Tokunaga M, et al. Phenotypic changes of lymphocytes in patients with systemic lupus erythematosus who are in longterm remission after B cell depletion therapy with rituximab. *J Rheumatol*. 2011;38:633-641.

70. Reis EA, Athanazio DA, Lima I, et al. NK and NKT cell dynamics after rituximab therapy for systemic lupus erythematosus and rheumatoid arthritis. *Rheumatol Int*. 2009;29:469-475.

71. Vallerkos T, Gunnarsson I, Widhe M, et al. Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. *Clin Immunol*. 2007;122:62-74.

72. Zhou H, Xu M, Qin P, et al. A multicenter randomized open-label study of rituximab plus rhTPO vs rituximab in corticosteroid-resistant or relapsed ITP. *Blood*. 2015;125:1541-1547.

73. Stasi R, Del Poeta G, Stipa E, et al. Response to B-cell depleting therapy with rituximab reverts the abnormalities of T-cell subsets in patients with idiopathic thrombocytopenic purpura. *Blood*. 2007;110:2924-2930.

74. Stasi R, Cooper N, Del Poeta G, et al. Analysis of regulatory T-cell changes in patients with idiopathic thrombocytopenic purpura receiving B cell-depleting therapy with rituximab. *Blood*. 2008;112:1147-1150.

75. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J Neuroimmunol*. 2006;180:63-70.

76. Cross AH, Klein RS, Piccio L. Rituximab combination therapy in relapsing multiple sclerosis. *Ther Adv Neurol Disord*. 2012;5:311-319.

77. Naismith RT, Piccio L, Lyons JA, et al. Rituximab add-on therapy for breakthrough relapsing multiple sclerosis: a 52-week phase II trial. *Neurology*. 2010;74:1860-1867.

78. Piccio L, Naismith RT, Trinka G, et al. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. *Arch Neurol*. 2010;67:707-714.

79. Studer V, Rossi S, Motta C, Buttari F, Centonze D. Peripheral B cell depletion and central proinflammatory cytokine reduction following repeated intrathecal administration of rituximab in progressive Multiple Sclerosis. *J Neuroimmunol*. 2014;276:229-231.

80. Bar-Or A, Fawaz L, Fan B, et al. Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? *Ann Neurol*. 2010;67:452-461.

81. Weber MS, Prod’homme T, Patarrroyo JC, et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B cell depletion in central nervous system autoimmunity. *Ann Neurol*. 2010;68:369-383.

82. Monson NL, Cravens P, Hussain R, et al. Rituximab therapy reduces organ-specific T cell responses and ameliorates experimental autoimmune encephalomyelitis. *PLoS ONE*. 2011;6:e17103.

83. Graves J, Vinayagasundaram U, Mowry EM, et al. Effects of rituximab on lymphocytes in multiple sclerosis and neuro-myelitis optica. *Mult Scler Relat Disord*. 2014;3:244-252.

84. Palanichamy A, Jahn S, Nickles D, et al. Rituximab efficiently depletes increased CD20-expressing T cells in multiple sclerosis patients. *J Immunol*. 2014;193:580-586.

85. Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*. 2002;346:1692-1698.

86. Gravellin M, Creswell L, Smith L, et al. Anti-CD20 monoclonal antibody rituximab in type 1 diabetes. *J Immunol*. 2011;187:1998-2005.
87. Wong FS, Wen L. B cells in autoimmune diabetes. Rev Diabet Stud. 2005;2:121-135.
88. Yadav D, Judkowski V, Fiodstrom-Tullberg M, et al. B7-2 (CD86) controls the priming of autoreactive CD4 T cell response against pancreatic islets. J Immunol. 2004;173:3631-3639.
89. Alunno A, Carubbi F, Bistoni O, et al. IL-17 producing pathogenic T lymphocytes co-express CD20 and are depleted by rituximab in primary Sjögren’s syndrome: a pilot study. Clin Exp Immunol. 2016;184:284-292.
90. Holley JE, Bremer E, Kendall AC, et al. CD20+ inflammatory T cells are present in blood and brain of multiple sclerosis patients and can be selectively targeted for apoptotic elimination. Mult Scler Relat Disord. 2014;3:650-658.
91. Prasad N, Jaiswal AK, Agarwal V, et al. Differential alteration in peripheral T-regulatory and T-effector cells with change in P-glycoprotein expression in Childhood Nephrotic Syndrome: a longitudinal study. Cytokine. 2015;72:561-566.
92. Lama G, Luongo I, Tirino G, Borriello A, Carangio C, Salsano ME. T-lymphocyte populations and cytokines in childhood nephrotic syndrome. Acta Paediatr Scand. 1989;78:87-93.
93. Prinza N, Papachristou F, Tziomouli V, Taparkou A, Kanakoudi-Tsakalidou F. Peripheral CD19+ B cells are increased in children with active steroid-sensitive nephrotic syndrome. NDT Plus. 2009;2:435-436.
94. Salsano ME, Graziano L, Luongo I, Pilla P, Giordano M, Lama G. Atopy in childhood idiopathic nephrotic syndrome. Acta Paediatr. 2007;96:561-566.
95. Yamashita H, Nakajima M, Naka H, et al. Up-regulation of interleukin-2 mRNA in children with idiopathic nephrotic syndrome. Acta Paediatr Scand. 1998;79:274-278.
96. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008;8:523-532.
97. Li X, Zheng Y. Regulatory T cell identity: formation and maintenance. Trends Immunol. 2015;36:344-353.
98. Dhaeze T, Stinissen P, Liston A, Hellings N. Humoral autoimmunity: a failure of regulatory T cells? Autoimmun Rev. 2015;14:735-741.
99. Araya CE, Wasserfall CH, Brusko TM, et al. A case of unfulfilled expectations. Cytokines in idiopathic minimal lesion nephrotic syndrome. Pediatr Nephrol. 2006;21:603-610.
100. Yan K, Nakahara K, Awa S, et al. The increase of memory T cells in patients with idiopathic minimal lesion nephrotic syndrome. Pediatr Nephrol. 2015;30:1365-1371.
101. Bertelli R, Bonanni A, Di Donato A, Cioni M, Ravani P, Ghiggeri GM. Regulatory T cells and minimal change nephropathy: in the midst of a complex network. Clin Exp Immunol. 2016;183:166-174.
102. Liu LL, Qin Y, Cai JF, et al. Th17/Treg imbalance in adult patients with minimal change nephrotic syndrome. Clin Immunol. 2011;139:314-320.
103. Le Berre L, Bruneau S, Naulet J, et al. Induction of T regulatory cells attenuates idiopathic nephrotic syndrome. J Am Soc Nephrol. 2009;20:57-67.
104. Madanchi et al. B cells and FOXP3 regulate T cells (Treg) in children with primary nephrotic syndrome. Pediatr Nephrol. 2009;24:1683-1690.
105. Roccia E, Sciaccia S, Di Simone D, et al. New insights into immune mechanisms underlying response to Rituximab in patients with membranous nephropathy: a prospective study and a review of the literature. Autoimmun Rev. 2016;15:529-538.
106. Holley JE, Bremer E, Kendall AC, et al. CD20+ inflammatory T cells are present in blood and brain of multiple sclerosis patients and can be selectively targeted for apoptotic elimination. Mult Scler Relat Disord. 2014;3:650-658.
107. Lama G. Atopy in childhood idiopathic nephrotic syndrome. Acta Paediatr Scand. 1998;79:274-278.
108. Prasad N, Jaiswal AK, Agarwal V, et al. Differential alteration in peripheral T-regulatory and T-effector cells with change in P-glycoprotein expression in Childhood Nephrotic Syndrome: a longitudinal study. Cytokine. 2015;72:190-196.
109. Yamashita H, Nakajima M, Naka H, et al. Up-regulation of interleukin-2 mRNA in children with idiopathic nephrotic syndrome. Pediatr Nephrol. 2004;19:1115-1121.
110. Prinza N, Papachristou F, Tziomouli V, Taparkou A, Kanakoudi-Tsakalidou F. IL-18 is correlated with type-2 immune response in children with steroid sensitive nephrotic syndrome. Cytokine. 2008;44:262-268.
111. Daniel V, Trautmann Y, Konrad M, Nayir A, Scharer K. T-lymphocyte populations, cytokines and other growth factors in serum and urine of children with idiopathic nephrotic syndrome. Clin Nephrol. 1999;62:575-580.
112. Yap HK, Cheung W, Morugas 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.
113. Chen SP, Cheung W, Heng CK, Jordan SC, Yap HK. Childhood nephrotic syndrome in relapse is associated with down-regulation of monocyte CD14 expression and lipopolysaccharide-induced tumour necrosis factor-alpha production. Clin Exp Immunol. 2003;134:111-119.
114. Acharya B, Shirakawa T, Pungky A, et al. Polymorphism of the interleukin-4, interleukin-13, and signal transducer and activator of transcription 6 genes in Indonesian children with minimal change nephrotic syndrome. Am J Nephrol. 2005;25:30-35.
115. 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.
116. Wei CL, Cheung W, Heng CK, et al. Interleukin-13 genetic polymorphisms in Singapore Chinese children correlate with long-term outcome of minimal-change disease. Nephrol Dial Transplant. 2005;20:728-734.
secretion of H(+)-and cathepsin L by glomerular epithelial
cells. Am J Physiol Renal Physiol. 2002;282:F26-F33.

120. Van Den Berg JG, Aten J, Chard MA, et al. Interleukin-4 and
interleukin-13 act on glomerular visceral epithelial cells. J Am Soc Nephrol. 2000;11:413-422.

121. Parry RG, Gillespie KM, Mathieson PW. Effects of type 2 cytokines on glomerular epithelial cells. Exp Nephrol. 2001;9:275-283.

122. Coers W, Vos JT, Van der Meide PH, Van der Horst ML, Huitema S, Weening JJ. Interferon-gamma (IFN-gamma) and IL-4 expressed during mercury-induced membranous nephropathy are toxic for cultured podocytes. Clin Exp Immunol. 1995;102:297-307.

123. Lai KW, Wei CL, Tan LK, et al. Overexpression of interleukin-13 induces minimal-change-like nephropathy in rats. J Am Soc Nephrol. 2007;18:1476-1485.

124. Simon D, Hosli S, Kostyлина G, Yawalkar N, Simon HU. Anti-CD20 (rituximab) treatment improves atopic eczema. J Allergy Clin Immunol. 2008;121:122-128.

125. Kim JE, Park SJ, Ha TS, Shin JI. Effect of rituximab in idiopathic nephrotic syndrome. Pediatr Nephrol. 2014;29:2207-2216.

126. Garin EH, Diaz LN, Mu W, et al. Urinary CD80 is elevated in minimal change patients in relapse increases CD80 expression in cultured podocytes. Pediatr Nephrol. 2015;30:309-316.

127. Garin EH, Diaz LN, Mu W, et al. Urinary CD80 excretion in idiopathic minimal-change minimal-change disease. J Am Soc Nephrol. 2009;20:260-266.

128. Garin EH, Fornoni A, Weins A, et al. Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black × New Zealand white mice. Response correlates with the absence of an anti-antibody response. J Immunol. 1996;157:3159-3164.

129. Reiser J, von Gersdorff G, Loos M, et al. Induction of B7-1 in podocytes is associated with nephrotic syndrome. J Clin Invest. 2004;113:1390-1397.

130. Garin EH, Mu W, Arthur JM, et al. Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis. Kidney Int. 2010;78:296-302.

131. Ling C, Liu X, Shen Y, et al. Urinary CD80 levels as a diagnostic biomarker of minimal change disease. Pediatr Nephrol. 2013;30:309-316.

132. Reiser J, von Gersdorff G, Loos M, et al. Induction of B7-1 in podocytes is associated with nephrotic syndrome. J Clin Invest. 2004;113:1390-1397.

133. Hoffman W, Lakkis FG, Chalasani G. B cells, antibodies, and more. Clin J Am Soc Nephrol. 2016;11:137-154.

134. Kairatidis L, Wang Y, Zheng L, Tay YC, Harris DC. Blockade of CD40-CD40 ligand protects against renal injury in chronic proteinuric renal disease. Kidney Int. 2003;64:1265-1272.

135. Lee VW, Qin X, Wang Y, et al. The CD40-CD154 co-stimulation pathway mediates innate immune injury in adriamycin nephrosis. Nephrol Dial Transplant. 2010;25:717-730.

136. Biancone L, Andres G, Ahn H, DeMartino C, Stamenkovic I. Inhibition of the CD40-CD40 ligand pathway prevents murine membranous glomerulonephritis. Kidney Int. 1995;48:458-468.

137. Hoffman W, Lakkis FG, Chalasani G. B cells, antibodies, and more. Clin J Am Soc Nephrol. 2016;11:137-154.

138. Kairatidis L, Wang Y, Zheng L, Tay YC, Harris DC. Blockade of CD40-CD40 ligand protects against renal injury in chronic proteinuric renal disease. Kidney Int. 2003;64:1265-1272.

139. Lee VW, Qin X, Wang Y, et al. The CD40-CD154 co-stimulation pathway mediates innate immune injury in adriamycin nephrosis. Nephrol Dial Transplant. 2010;25:717-730.

140. Biancone L, Andres G, Ahn H, DeMartino C, Stamenkovic I. Inhibition of the CD40-CD40 ligand pathway prevents murine membranous glomerulonephritis. Kidney Int. 1995;48:458-468.

141. Early GS, Zhao W, Burns CM. Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black × New Zealand white mice. Response correlates with the absence of an anti-antibody response. J Immunol. 1996;157:3159-3164.

142. Reiser J, von Gersdorff G, Loos M, et al. Induction of B7-1 in podocytes is associated with nephrotic syndrome. J Clin Invest. 2004;113:1390-1397.

143. Garin EH, Mu W, Arthur JM, et al. Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis. Kidney Int. 2010;78:296-302.

144. Ling C, Liu X, Shen Y, et al. Urinary CD80 levels as a diagnostic biomarker of minimal change disease. Pediatr Nephrol. 2013;30:309-316.

145. Garin EH, Diaz LN, Mu W, et al. Urinary CD80 excretion increases in idiopathic minimal-change minimal-change disease. J Am Soc Nephrol. 2009;20:260-266.

146. van de Veerdonk FL, Lauwerys B, Marijnissen RJ, et al. The anti-CD20 antibody rituximab reduces the Th17 cell response. Arthritis Rheum. 2011;63:1507-1516.

147. Rizk MK, El-Nawawy A, Abdel-Kareem E, Amer ES, El-Gezairy D, El-Shafei AZ. Serum interleukins and urinary microglobulin in children with idiopathic nephrotic syndrome. East Mediterr Health J. 2005;11:993-1002.

148. Garin EH, Blanchard DK, Matsushima K, Djeu JY. IL-8 production by peripheral blood mononuclear cells in nephrotic patients. Kidney Int. 1994;45:1311-1317.

149. Souto MF, Teixeira AL, Russo RC, et al. Immune mediators in idiopathic nephrotic syndrome: evidence for a relation between interleukin 8 and proteinuria. Pediatr Res. 2008;64:637-642.

150. Garin EH, Blanchard DK, Matsushima K, Djeu JY. IL-8 production by peripheral blood mononuclear cells in nephrotic patients. Kidney Int. 1994;45:1311-1317.

151. Souto MF, Teixeira AL, Russo RC, et al. Immune mediators in idiopathic nephrotic syndrome: evidence for a relation between interleukin 8 and proteinuria. Pediatr Res. 2008;64:637-642.

152. Matsunoto K, Kanmatsuse K. Increased IL-12 release by monocytes in nephrotic patients. Clin Exp Immunol. 1999;117:361-367.

153. Kilis-Pstrusinska K, Medynska A, Zwolinska D, Wawro A. Interleukin-18 in urine and serum of children with idiopathic nephrotic syndrome. Kidney Blood Press Res. 2008;31:122-126.

154. Li L, Zhao YW, Zeng JS, et al. Rituximab regulates the expression of the Raf kinase inhibitor protein via NF-kB in renal tissue of rats with diabetic nephropathy. Genet Mol Res. 2013;12:2973-2981.

155. Maas RJ, Deegens JK, Wetzel JS. Permeability factors in idiopathic nephrotic syndrome: historical perspectives and lessons for the future. Nephrol Dial Transplant. 2014;29:2207-2216.

156. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13:227-242.
155. Bitzan M, Babayeva S, Vasudevan A, Goodyer P, Torban E. TNFα pathway blockade ameliorates toxic effects of FSGS plasma on podocyte cytoskeleton and β3 integrin activation. Pediatr Nephrol. 2012;27:2217-2226.

156. Bollinger CR, Teichgraber V, Gulbins E. Ceramide-enriched membrane domains. Biochim Biophys Acta. 2005;1746: 284-294.

157. Yoo TH, Pedigo CE, Guzman J, et al. Sphingomyelinase-like phosphodiesterase 3b expression levels determine podocyte injury phenotypes in glomerular disease. J Am Soc Nephrol. 2015;26:133-147.

158. d’Hennezel E, Piccirillo CA. Analysis of human FOXP3+ Treg cells phenotype and function. Methods Mol Biol. 2011;707:199-218.

159. Samuel S, Scott S, Morgan C, et al. The Canadian Childhood Nephrotic Syndrome (CHILDNEPH) Project: overview of design and methods. Can J Kidney Health Dis. 2014;1:17.