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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.

Abrégé

Le traitement par le rituximab, un anticorps monoclonal contre la protéine de surface CD20 des lymphocytes B, mène à l'appauvrissement de ces derniers. Le rituximab a récemment été signalé comme étant efficace pour prévenir les rechutes des maladies à changements minimes (MCD) dépendantes des glucocorticoïdes ou des MCD à rechutes fréquentes. On pense que les MCD pourraient être induites par les lymphocytes T, mais la manière dont le rituximab agit sur les MCD n'est pas encore bien comprise. Dans cette revue, nous résumons les principales études cliniques démontrant l'efficacité du rituximab dans le syndrome néphrotique idiopathique, principalement dans les MCD. Nous discutons ensuite des caractéristiques immunologiques de la maladie et des mécanismes d'action potentiels du rituximab dans son traitement, en nous basant sur ce que l'on connaît de l'action thérapeutique du rituximab dans d'autres troubles immunitaires. Nous sommes d'avis que des études visant une meilleure compréhension des mécanismes d'action du rituximab dans les MCD pourront fournir de nouvelles approches pour remédier à la pathophysiologie immunitaire des MCD.

Keywords

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

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Why is this review important

It provides a framework for future studies directed at understanding the pathogenesis of minimal change disease (MCD), which has remained elusive.

What are the key messages

Studying how rituximab works in MCD will help elucidating the pathogenesis of MCD.

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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.[1] The sole histological abnormality is the disappearance of podocyte foot processes (effacement) that is detected by electron microscopy.[1] 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,[4] 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).[5] Moreover, about 10% to 20% of the patients show primary or late glucocorticoid resistance (steroid-resistant nephrotic syndrome [SRNS]).[5,6]

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.[7] 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.[4,8] 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.[9] 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,[10-14] 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.[15] Ravani 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.[16,17] In another RCT by Ravani et al,[18] 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.[19] Overall, there is strong evidence to support the efficacy of RTX in glucocorticoid responsive MCD/INS in children.

Table 1. Studies on the Effects of RTX in Patients With INS.

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	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. 3.7%; 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: 0-1; P < .001), prednisone maintenance dose from 0.27 mg/kg (IQR: 0.19-0.60) to 0 mg/kg (IQR: 0-0.23; P < .001), and the median cumulative dose of GC to maintain remission from 19.5 mg/kg (IQR: 13.0-29.2) to 0.5 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 < .0001)
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)

Table 1. (continued)

Study	Patient characteristics	Methodology	Results
Ito et al[25]	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[26-28]	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[27]	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 FRNS/SDNS and SRNS patients ($P = .175$)
Bagga et al[26-28]	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[29]	4 children with SRNS (2 FSGS, 1 IgM nephropathy, 1 MCD). All were negative for NPHS2 gene mutation NPHS2 gene mutation (encoding podocin)	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[30]	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/ m^2	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-Fresnedo et al[31]	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-analysis[21] is a retrospective multicenter study by Guitard et al[22] who reported complete or partial remission with cessation or reduction of other immunosuppressants 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,[30] 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

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.[51-53] Another study failed to observe T-cell depletion[54] 2. Inhibition of CD4+ T-cell activation and inhibition of T cells and Mφ in synovial tissue[55] 3. Decreased RANKL expression in synovium and serum[56] suggesting inhibition of Th cell activity 4. Decreased Mφ counts and activity[53,55,57,58] 5. Decreased cytotoxic lymphocytes and mature NK cells and a simultaneous upregulation of activated NK cells[59,60] 6. Decreased IL-15 and IL-17. Clinical improvement may be associated with IL-15/memory T-cell–related mechanisms.[61] (IL-15 is known to expand and activate NK cells.) 7. Decreased IL-2, IL-6, IL-7, and IL-10 serum levels[62] 8. Decreased production of IFN-γ and IL-1β by T cells[55] 9. Downregulation of genes involved in immunoglobulins, chemotaxis, leukocyte activation, and immune responses with upregulation of genes involved in TGF-β pathway in synovial tissue[63] 10. Decreased MMP-1, MMP-3, MMP-9 in serum[64] likely related to decreased Mφ count/activity
SLE	1. Possible inhibition of T-cell co-stimulation[65-69] 2. Decreased memory T cells relative to naive T cells[69] 3. Decreased expression of CD19, CD21, and BR3 (receptor for the B-cell activating cytokine B-cell activating factor, BAFF, BAFF) on the residual B cells and after B-cell recovery[65] 4. Decreased percentage of CD69+CD4+T cells (activated T cells) and serum TNF-α level[65] 5. Shift of the Th1/Th2 balance toward Th1[65] 6. Increased Treg cell number and their regulatory function[66] 7. Increased activated NK and natural killer T cells[70] 8. Increased activated CD4 and CD8 T cells, in contrast to other studies[71]
ITP	1. Immediate response to RTX while autoantibodies are still present[72] 2. Normalized increased Th1/Th2 ratio[73] 3. Restored number and regulatory function of Treg cells[74]
MS	1. Therapeutic effects without changes in serum or CSF antibody levels[75-79] 2. Decreased T-cell number and proinflammatory cytokines in CSF[75-82] 3. Reduction in Th1 and Th17 cells and diminished proinflammatory responses of both CD4 (Th1 and Th17) and CD8 T cells mediated by B-cell lymphotoxin and TNF-α secretion[80] 4. Inhibition of B-cell–dependent T-cell proliferation and Th17 differentiation[82] 5. Transient decrease in CD4+ and CD8+ T cells in blood[83] 6. Depletion of increased CD20dim T cells (either CD4+ or CD8+)[84]
DM1	1. Although anti–T-cell therapies (eg, anti-CD3 antibody) are effective in treatment of DM1,[85] RTX was also shown to be helpful in new-onset DM1[86] 2. Reduction in autoantibody production or autoreactive B-cell counts[87] 3. Increased Treg cells in short term[86] 4. Modification of the B7-2/CD28 co-stimulation pathway that plays a critical role in priming islet-reactive Th cells[88]

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.

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.[48] In MCD, post-RTX relapses often occur with B-cell recovery, likely an indication of a pathogenic B-cell population resurgence.[49] 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,[49] or postulated direct effects of RTX on podocytes.[50] 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

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.[51-53,65,75,76,80,81,83] In addition, a small, likely pathogenic subset of T cells has been identified in patients with RA; they express small amounts of CD20 ($CD20^{dim}$) and are targeted by RTX. These cells are characterized by a shift from Th1 to Th17 subtype, associated with T-cell–mediated inflammation.[89] A fraction of IL-17–producing $CD3^+CD4^-CD8^-$ ("double negative") T cells and $CD4^+$Th17 cells co-express CD20 on the cell surface and are depleted by RTX in primary Sjogren's syndrome.[90] A similar phenomenon has been demonstrated for patients with MS.[84,91]

Reports describing the distribution of B-cell and T-cell subsets in MCD are limited and show variable results. Estevez et al found increased peripheral B-cell counts in 23 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.[92] 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.[93,94] Lama et al noted a decreased proportion of $CD4^+$ cells both in children with SSNS in relapse and SRNS,[95] 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.[96] 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.[97]

According to a model proposed by Lund and Randall,[48] 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).[98] Treg cells function as immune suppressors through several mechanisms, including the production of inhibitory cytokines, IL-10, and TGF-β.[99] 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.[99] 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.[100]

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,[101] 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.[96,102-105] 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.[96,102-104] 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.[106] 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.[96,102-104] Shao et al suggested a role for a dynamic equilibrium between Th17 (effector) and Treg cells in patients with various glomerulopathies.[104] Interestingly, in the Buffalo/Mna rat model of FSGS, Treg cells were increased in drug-induced remission, and transfer of Treg cells induced remission.[107] 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.[66,74] 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.[108] 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 patients[95,96,110]; 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.[113] 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 and fusion of podocyte foot processes in IL-13 overexpressing rats, associated with increased glomerular expression of CD80, IL-4R, and IL-13R2 and downregulation of nephrin, podocin, and dystroglycan expression.[123] Although these findings are intriguing, they have not been validated by others. Nonetheless, RTX has been shown to reduce IL-13 levels in patients with atopic eczema,[124] suggesting that the action of RTX in MCD may involve the reduction of IL-13 expression.[125]

Modulations of Other T-Cell Subsets and Cytokines

Th17 cells. Liu et al noted a significant increase in Th17 cell numbers as well as plasma levels of Th17-related cytokines, IL-17 and IL-23, in MCD patients.[106] Recombinant murine IL-17 exerts a proapoptotic effect in cultured mouse podocytes.[126] Th17 cells may play a role in the pathogenesis of MCD. RTX treatment was associated with a decrease in Th17 cells and Th17-related cytokines (particularly IL-17) in affected tissues and in the circulation of patients with primary Sjogren's syndrome[127] and RA.[128] It is tempting to speculate that Th17 cells play a role in the pathogenesis of MCD and that the reduction of Th17 cells is one of the actions of RTX in MCD.

Other cytokines and possible permeability factors. Other cytokines have been reported to be increased in biological samples from MCD patients during relapse of proteinuria, many of which decreased during remission, for example, IL-1 and IL-6,[129] IL-2,[95,110,111,113] IL-8,[130,131] IL-12,[132] and IL-18.[112,133] 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.[62] 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.[150] 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.[151] 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,[138,152] and its pathological role was suggested in post-transplant recurrence of FSGS, although the evidence was indirect.[152]

Role of Antigen Presentation by B Cells

B cells present antigens to T cells.[153] 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.[49] 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 $CD4^+/CD8^+$ ratio.[95,113] However, data on a possible role of $CD8^+$ T cells in MCD are scarce. Natural killer (NK) cell deficiency was associated with frequently replacing MCD in a patient with Hodgkin's disease,[154] and RTX was shown to increase active NK cells in patients with RA and SLE,[59,60,70] suggesting a possible implication of NK cells in the effect of RTX in MCD. However, another study reported functionally normal NK cells in MCD.[92] RTX was also shown to decrease the number of synovial macrophages (Mφ)[53] and to inhibit TNF-α production by Mφ in patients with RA.[57] Although the data suggest a role for TNF-α in some patients with recurrent FSGS post-transplantation,[155] 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.[50] SMPDL-3b and ASMase are essential for the organization of receptors and signaling molecules in highly specialized cells[156] and are involved in the modulation of actin remodeling in podocytes.[50] 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.[50] Yoo et al suggested that SMPDL-3b may be an important modulator of podocyte function and a novel therapeutic target in glomerular diseases.[157] 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.[135] 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 $CD20^+$ T cells[91] or effector B cells.[135] 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.[158] 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.[159]

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; Mφ, macrophages; MS, multiple sclerosis; NK cells, 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)–sensitive nephrotic syndrome; TGF-β, transforming growth factor-β; Th, 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.

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Not applicable.

Consent for Publication

All authors have consented for article publication.

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Not applicable.

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