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Review

New insights into immune mechanisms underlying response to RTX in patients with membranous nephropathy: A prospective study and a review of the literature

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ABSTRACT

Background: Idiopathic membranous nephropathy (MN) is a common immune-mediated glomerular disease and the main cause of nephrotic syndrome (NS) in Caucasian adults. Rituximab (RTX) has been reported to safely reduce proteinuria in patients with primary MN and severe NS. However, the effects of RTX treatment on T–cells including regulatory T cells (Treg) in MN have not been fully determined.
Methods: Seventeen patients [mean age 67 (29–86) years, 6 women, 11 men] with biopsy-proven MN, and persistent proteinuria >3.5 g/24 h were prospectively enrolled and received RTX, 375 mg/m 2 (iv) on days 1, 8, 15 and 22. Changes in circulating B and T cell homeostasis were examined in the peripheral blood by flow-cytometry studies; serum levels of IL-35 were measured using a high-sensitivity ELISA kits (baseline, at month 3, 6, 9 and 12).
Results: Patients had been followed-up for a mean of 36.3 months (24–48) months. Proteinuria decreased from 5.6 (3.5–8) g/24 h to 2.4 (0.06–13) g/24 h at 6 months ($p < 0.05$) and to 1.3 (0.06–8) at 12 months ($p < 0.01$), respectively after therapy with RTX. Four patients received a 2nd course of RTX (one at 6 months because of persistent NS, and three at 12, 18, or 30 months for relapse). The three relapsing patients became proteinuria-free (<0.5 g/24 h) in the following 6 months. Serum creatinine remained stable during the follow-up: median 1 mg/dl (0.7–1.6) at 12 months and 1.1 (0.7–1.7) at 24 months as compared to 1 (0.5–2.4) at baseline. At 6 months after RTX, complete remission (CR) was observed in 7 patients, partial remission (PR) in 4 and non responder (NR) in 6. At the end of the follow-up, 14 patients were in CR, 1 in PR and NR 2. In the T cell compartment, upon detection of B cell depletion, there was an increase in Treg up to 10-fold when comparing baseline and at month 12 (mean ± SD 1.2 ± 0.6%, and 5.8 ± 0.7% $p = 0.02$, respectively). When stratifying patients in responders (CR + PR) and NRs at month 12, we observed a significant increase in Treg cells from month 6 which persisted till 12 months only in the responder group (5.5 ± 0.6% and 1.1 ± 0.6%, $p = 0.04$, respectively in responders and NRs). A statistically significant decrease in the levels of active T-lymphocytes (HLA-DR + CD8 + cells) was observed, with a maximum reached at 12 months after treatment with RTX [6 ± 1.1% baseline, 4.7 ± 1.7% at 6 months ($p = 0.043$) and 1.5 ± 1.4% at 12 months ($p = 0.05$)]. A marked increase in IL-35 levels [defined as delta >40% (serum values at 6 months minus baseline values)] was seen in 68% of the patients who achieved clinical response (CR or PR) at 12 month, but in none of the patients who failed to respond ($p = 0.034$).
Conclusion: Our findings and data from literature support the idea that RTX can be envisaged as a first-line therapy for patients at risk of progression because of persistent NS due to idiopathic MN. Insights into the putative T cell-related mechanisms of action have been discussed.

Contents

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1. Introduction

Idiopathic membranous nephropathy (MN) is a common immune-mediated glomerular disease and remains as the main cause of nephrotic syndrome (NS) in Caucasian adults [1–5]. Although in most patients the disease progresses relatively slowly, about 40% of patients eventually develop end-stage renal disease (ESRD) [6,7]. Available immunosuppressive therapies include the use of corticosteroids combined with cytotoxic agents, and calcineurin inhibitors. These therapies are at least partially successful in reducing proteinuria, but their use is controversial, associated with significant adverse effects and carries a high rate of relapse (reviewed in [3,8,9]). These are important considerations in a disease where up to 30% of MN patients may achieve spontaneous remission of proteinuria with long-term renal survival with only supportive therapy [10].

Major advances in understanding the pathophysiology of MN have occurred since the early 2000s with the identification of neutral endopeptidase as the first human podocyte antigen involved in a rare subset of patients with neonatal alloimmune MN [11,12], followed by the characterization of phospholipase A_2 receptor (PLA_2R), another podocyte antigen targeted by circulating antibodies in 70%–80% of adult patients with primary MN. This major breakthrough showed that primary MN is an autoimmune disease in which the podocyte is the target and the source of the autoantigen [13].

Starting from these assumptions, in 2002, rituximab (RTX), a monoclonal antibody against the cell surface antigen CD20 of B cells [14–20], was reported to safely reduce proteinuria and ameliorate NS in 8 patients with primary MN and severe NS unresponsive to prolonged angiotensin-converting-enzyme (ACE) inhibitor therapy [21]. Subsequent studies consistently confirmed these preliminary findings [22] even when RTX was administered as a second-line treatment in patients who had previously failed to respond to steroids, alkylating agents, or calcineurin inhibitors or who had relapsed after transient remission [23,24]. Finding that RTX therapy achieved disease remission and stabilized or even improved renal function in 100 patients at high risk of poor outcomes because of persistent NS pointed to a pathogenic role of antibody-producing lymphocytes in primary MN [25]. Indeed, experimental and human data converge to indicate that deposition along the glomerular basement membrane of immunoglobulins produced by autoreactive B cells initiates the sequence of events resulting in secondary injury to the glomerular filtering barrier and proteinuria [26]. Therefore, agents that specifically interfere with B cell antibody production would ideally be the first step toward selective therapy for primary MN.

Recently, successful treatment with RTX has been associated with regression of some T-cell abnormalities in patients with autoimmune conditions such as chronic ITP [27] and SLE [15]. Consistently, data from our group showed a T-lymphocytes re-assessment after RTX in patients with severe lupus nephritis [15]. However, the effects of RTX treatment on T–cells including regulatory T cells (Treg) in MN have not been fully determined.

These considerations prompted us to critically review the most recent literature about the about the role of T-lymphocytes in MN pathogenesis. Similarly, in order to further define immune mechanisms underlying response to B-cell depletion, we prospectively assessed lymphocytes re-assessment (including CD4 + FOXP3 + Tregs) and IL-35 levels as predictors of response to RTX in patients with MN. Additionally, the observed clinical outcome results were compared to those emerging from the updated reviews of the literature on this topic.

2. Predictors of response in MN patients treated with RTX

Given the variability in the natural history of the disease, historically, an approach in MN has been to limit immunosuppression treatment to those subjects identified as being at higher risk of progression, and a number of predictors of renal outcome and disease progression have been identified for patients with idiopathic MN [28,29]. However, in treated patients, little is known regarding factors that may predict response to therapy, especially in regard to the use of RTX.

Few studies have evaluated the use of urinary markers for this purpose. Bazzi et al. [30] quantified urinary IgG and α1-microglobulin in 38 patients with NS and normal renal function. Using an arbitrary cutoff value, these authors showed that 100% of patients with a baseline IgG excretion of <110 mg/g urinary creatinine (uCr) underwent remission of proteinuria versus 20% in those with an IgG excretion of >110 mg/g uCr. Similarly, 77% of the patients with an α1-microglobulin of <33.5 mg/g uCr went into remission versus 17% of the patients with an α1-microglobulin of >33.5 mg/g uCr. Of note, the remission rate was independent of baseline proteinuria. Of the 38 patients, 19 were allocated, in a non-randomized fashion, to receive either corticosteroids and cyclophosphamide for 6 months ($n = 16$) or corticosteroids alone

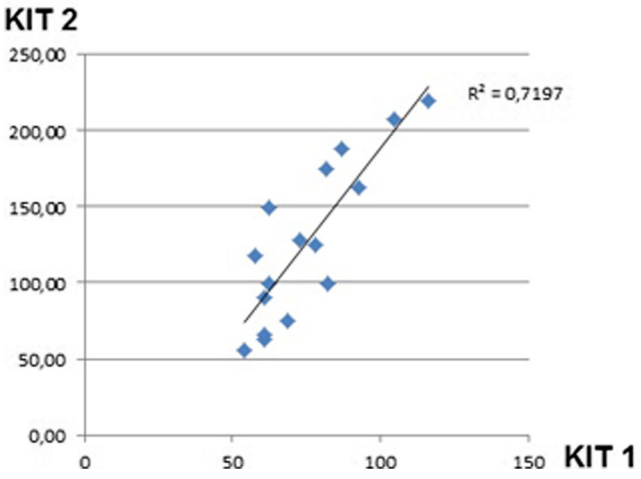

Fig. 1. Correlation analysis by Spearman test for Kit A and Kit B used for IL-35 detection.

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