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Exceptional Case

Homozygous FCGR3A-158F mutation is associated with delayed B-cell depletion following rituximab but with preserved efficacy in a patient with refractory lupus nephritis

Guillaume Seret[1,2,3], Catherine Hanrotel[3], Boutahar Bendaoud[1,2], Yannick Le Meur[1,3] and Yves Renaudineau[1,2]

[1]EA2216 Immunology and Pathology, European University of Brittany, Brest, France, [2]Laboratory of Immunology, Brest University Medical School, CHRU Morvan, Brest, France and [3]Unit of Nephrology, Brest University Medical School, CHRU Cavale Blanche, Brest, France

Correspondence and offprint requests to: Yves Renaudineau; E-mail: yves.renaudineau@univ-brest.fr

Abstract

Rituximab (RTX), an anti-CD20 monoclonal antibody, has shown promising results in a small group of systemic lupus erythematosus (SLE) patients treated for lupus nephritis (LN). However, such observations were not confirmed in the double-blind LUNAR study. Accordingly, the factors associated with the clinical response remain to be characterized. We report the case of a young woman with known LN successfully re-treated with RTX and steroids and homozygous for the low-affinity FCG3RA 158F genotype. Although B-cell depletion was delayed, complete remission with anti-DNA antibody negativity and proteinuria normalization were maintained for 5 years. The implications for disease pathogenesis and clinical monitoring are discussed.

Keywords: B-cell depletion; FCGR3A; lupus nephritis; rituximab

Background

Lupus nephritis (LN) is a severe and common complication of systemic lupus erythematosus (SLE). The conventional treatment with steroids and immunosuppressants leads to complete or partial remission. However, in up to 50% of cases, despite high doses of immunosuppressants, LN is not controlled and relapse may occur. For these patients, B-cell depletion therapy using rituximab (RTX, Mabthera™) appears to be a promising treatment, and has been proven to be beneficial in a small group of patients [1]. However, in the LUpus Nephritis Assessment with Rituximab (LUNAR) study, RTX did not improve the clinical outcomes after 1 year [2]. Thus, RTX seems to be effective in a fraction of LN patients but it needs to be characterized.

Case report

The diagnosis of SLE with cutaneous location was established, in February 2000, in a 31-year-old woman. Nephritic syndrome appeared a month later with proteinuria at 5 g/24 h without renal failure and she was treated with oral steroids and pulsed intravenous cyclophosphamide. A renal biopsy revealed a proliferative glomerulonephritis type IV-G (A/C). In 2002, the first LN flare was treated with steroids and mycophenolate mofetil, and the second flare, in 2004, led to the initial treatment with RTX at 350 mg/m^2 weekly for 4 weeks leading to a complete remission. In April 2006, the patient stopped all medical treatments and follow-up.

On admission in April 2007, the patient presented with acute renal failure combined with nephritic syndrome and generalized oedema. The blood pressure was 121/87 mmHg, and the plasma albumin had fallen to 7.5 g/L with a weight gain of 19 kg. Proteinuria at 6.6 g/24 h was associated with a microscopic haematuria. The renal biopsy was unchanged. Laboratory examination revealed changes in the complement classical pathway (C3c: 0.19; normal range (NR) 0.69–1.34 and C4: 0.07; NR: 0.14–0.33), anti-nuclear antibody (Ab) positivity (1/640; NR < 1/160) and anti-dsDNA Ab positivity (dsDNA ELISA 0.749, NR < 0.200). Anti-dsDNA Ab cross-reactivity with alpha-actinin (0.252; NR < 0.170) and anti-C1q Ab were detected (0.743; *N* < 0.400).

Based on the effectiveness of RTX in this patient, a new course of RTX was initiated at 350 mg/m^2 weekly for 4 weeks. In order to improve its efficacy, the first injection of RTX was associated with pulses of 1 g methylprednisolone at Days 1, 2 and 3 and followed by oral steroids (60 mg/day) which were progressively tapered (10 mg/day at 6 months).

Complete remission, defined as a decrease in proteinuria <0.5 g/24 h, disappearance of haematuria and normalization of creatinaemia, was achieved at 32 weeks and maintained through 5-year follow-up (Figure 1A). The complement fractions C3c and C4 were normalized at

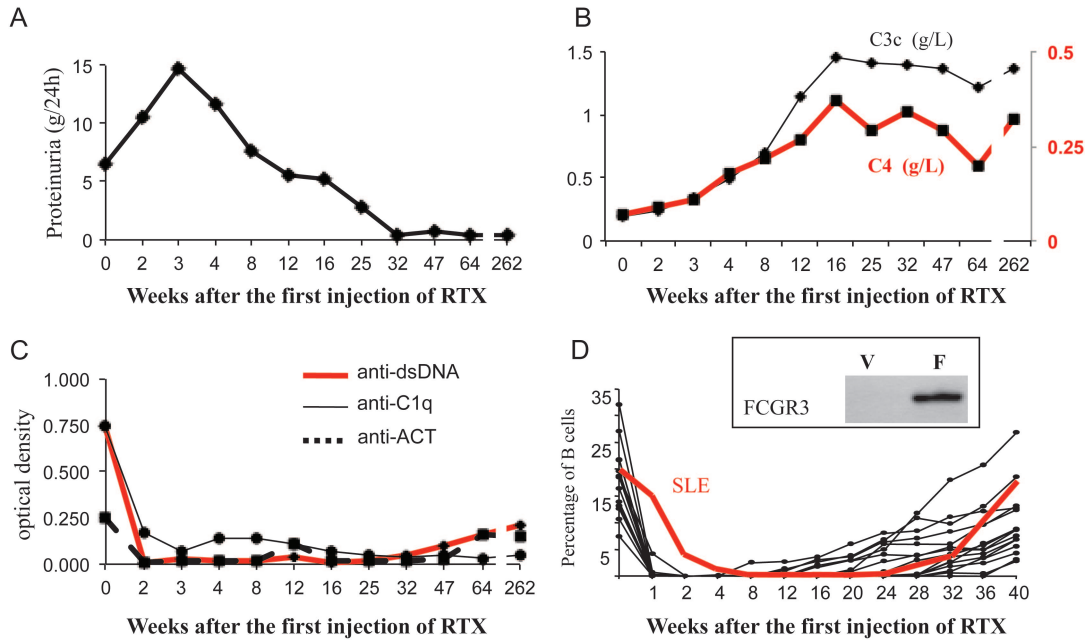

Fig. 1. Longitudinal variations of (**A**) proteinuria; (**B**) C3c and C4 complement fractions; (**C**) anti-dsDNA Ab, anti-C1q Ab and anti-alpha actinin (ACT) Ab and (**D**) percentage of peripheral blood B cells in a lupus nephritis patient re-treated with RTX and compared with 14 primary Sjögren's syndrome patients treated with RTX. The 14 SS patients displayed the three FCGR3A genotypes (5 were F/F, 9 were V/F and 1V/V). Of particular note, the SS patient who achieved B-cell depletion at 2 weeks was FCGR3A 158F/F. In the figure, allele-specific PCR is showing an FCGR3A 158F/F genotype.

1 month (Figure 1B). Within 2 weeks, anti-dsDNA/α-actinin Ab and anti-C1q Ab were undetectable (Figure 1C).

In contrast to 14 primary Sjögren's syndrome (pSS) patients used as controls who achieved B-cell depletion (<0.1% circulating B cells) at first or at second infusion [3], B-cell depletion was delayed to the fourth infusion for the reported case (Figure 1D). In an attempt to understand such phenomena, analysis was performed for the FCGR3A 158V/F polymorphism by allele-specific PCR revealing a low-affinity FCGR3A 158F/F phenotype. Human anti-chimeric antibodies were undetectable during the follow-up. Interestingly, the difficulty in achieving complete B-cell depletion did not influence the time of reappearance of B cells when compared with the pSS control group.

Discussion

In this case report, we present re-treatment of a refractory FCGR3A F/F LN Type IV patient with RTX, which resulted in a complete remission of up to 5 years after the first injection. No side effects or adverse events were noticed.

Although anti-dsDNA/actinin Ab and anti-C1q Ab were undetectable from the second week, it was not until the fourth week that peripheral blood B-cell depletion was achieved. As a consequence, it could be hypothesized that auto-Ab-producing B cells are particularly sensitive to the action of RTX. This observation is in line with previous observations showing a correlation between an anti-dsDNA Ab reduction and the biological activity of RTX [4, 5]. However, such an effect on the anti-dsDNA Ab may not predict remission in all LN patients. Indeed, the contribution of anti-dsDNA Ab to glomerulonephritis is not completely understood, since it may sometimes target glomerular antigens by cross-reaction, while at other times it may form immune complexes with nucleosomes.

As demonstrated in SLE [6], the low-affinity genotype FCGR3A 158F/F delays B-cell depletion which was the case in the present report. *In vitro*, FCGR3A 158F/F NK cells are 4.2-fold less effective than 158V/V NK cells in inducing antibody-dependent cellular cytotoxicity (ADCC) [7]. As a consequence, the FCGR3A genotype correlates with the RTX clinical outcomes of rheumatoid arthritis [8] and non-Hodgkin's lymphoma, but not in those with chronic lymphocytic leukaemia [9]. In SLE, the proof remains to be established.

Now the standing question is, how do we use RTX as an alternative to the standard treatment in refractory LN patients or in patients who experience a new flare after immunosuppressive treatment? The combination with steroids, suspected to be beneficial, also needs further evaluation in controlled trials. Several criteria have been presented to predict a better response to RTX such as a Type III LN, the absence of nephritic syndrome and renal failure, an active renal disease and a first response to RTX [10]. In addition, pharmacogenetic markers such as FCGR3A and serological normalization may also be helpful. We believe that our observation may be useful both in designing trials with biotherapy and in considering re-treatment in LN patients.

Conflict of interest statement. None declared.

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