EJR European Journal of Rheumatology
DOI: 10.5152/eurjrheum.2019.18109

Original Article

Rituximab levels are associated with the B cell homeostasis but not with the clinical response in patients with rheumatoid arthritis

Cèsar Díaz-Torné[1], Maria Àngels Ortiz[2], Mónica Sarmiento[1,3], César Díaz-López[1], Hèctor Corominas[1], Jordi Casademont[4], Silvia Vidal[2]

Abstract

Objective: To study the levels of rituximab (RTX) and anti-RTX antibodies (ARAs) in patients with rheumatoid arthritis (RA) at 30, 90, and 180 days after the first infusion, in relation to clinical and serological parameters and B cell homeostasis.

Methods: Thirty-four patients with RA who failed to respond to anti-tumor necrosis factor therapy received RTX. At baseline, 4, 12, and 24 weeks after the first infusion of RTX, we performed a clinical assessment and determined the levels of RTX, ARAs, B cells, rheumatoid factors, anti-cyclic citrullinated peptide antibodies, immunoglobulins, and complements.

Results: RTX levels varied widely among patients. No ARAs were detected during the follow-up. Patients with lower levels of RTX presented with higher decreases in erythrocyte sedimentation rate, immunoglobulins, and complement 6 months after the first infusion. Patients with higher levels of RTX showed a higher B cell depletion at 90 days but an earlier B cell recovery than those with lower levels of RTX. No differences in clinical response were observed between the two groups at 6 months after starting the treatment.

Conclusion: Our findings suggest that RTX levels in the serum of patients with RA are related to B cell homeostasis and the severity of immunological parameters but not to the clinical response at 6 months.

Keywords: Rheumatoid arthritis, rituximab, B cells

ORCID IDs of the authors:
C.D.T. 0000-0001-6275-7699;
M.A.O. 0000-0002-1574-9861;
M.S. 0000-0001-5212-9904;
C.D.L. 0000-0002-8416-1068;
H.C. 0000-0002-7738-6787;
J.C. 0000-0002-8100-1827;
S.V. 0000-0002-3909-6682.

Cite this article as: Díaz-Torné C, Ortiz MA, Sarmiento M, Díaz-López C, Corominas H, Casademont J, Vidal S. Rituximab levels are associated with the B cell homeostasis but not with the clinical response in patients with rheumatoid arthritis. Eur J Rheumatol 2019; 6(2): 81-4.

[1] Department of Rheumatology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[2] Department of Immunology, IIB-Institut de Recerca de l'Hospital de Sant Pau, Barcelona, Spain
[3] Department of Rheumatology, Colmédica, Bogota, Spain
[4] Departament of Internal Medicine, Hospital de la Santa Creu i Sant Pau. Universitat Autònoma de Barcelona, Barcelona, Spain

Address for Correspondence: Cèsar Díaz-Torné; Department of Rheumatology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
E-mail: cesardiaztorne@gmail.com

Submitted: 5 July 2018
Accepted: 18 January 2019

Introduction

Advances in knowledge of rheumatoid arthritis (RA) have shown that this disease is not a syndrome as different subsets of patients have different prognoses and responses to treatment. Our greater understanding of the pathogenesis of the disease has also led to the development of biological therapies. One such therapy is rituximab (RTX), a glycosylated chimeric mouse/human monoclonal antibody directed toward CD20, a pan-B surface marker that depletes B lymphocytes. B cell depletion after RTX treatment differs in distinct diseases, tissues, and from patient to patient (1-2). As in other diseases, such as lymphoma (3), and with other biological therapies (4), higher serum levels of RTX should predict a better B cell depletion and clinical response. However, to date, studies of RTX levels in patients with RA have not found an association between RTX levels and clinical outcomes. None of these studies completed the B cell count or determined RTX levels beyond 3 months. We prospectively analyzed clinical outcomes and B cell and RTX levels in an RA cohort during a complete course of treatment.

Methods

Peripheral blood samples were obtained from 34 patients who met the American College of Rheumatology criteria for RA. Treatment with RTX was started following standard clinical practice. All patients were refractory to treatment with synthetic disease-modifying antirheumatic drugs, including methotrexate, and tumor necrosis factor antagonists. The study was approved by the ethics committee of the Hospital de Sant Pau according to the Declaration of Helsinki. Written consent was obtained from all patients. Heparinized blood samples were collected on days 0 (baseline, before treatment), 30, 90, and 180 days after the first RTX infusion. Every course cycle consisted of two doses of RTX (1000 mg) 2 weeks apart. Nine patients had minor infusion reactions that required a slower infusion rate, but completed

treatment. One patient presented a major infusion. Treatment was terminated, and the patient was excluded. At all visits, we determined erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), immunoglobulins (IgG, IgA, and IgM), complements (C3 and C4), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (ACPAs), and RTX and anti-RTX levels.

Flow cytometry

Venous blood was processed for flow cytometry as described previously, within 2 h of collection (5). Monoclonal antibodies used were as follows: Cyto-STAT tetrachrome system lymphocyte cocktail (anti-CD45-FITC/CD4-phycoerythrin (PE)/CD8-PE-Texas red (ECD)/CD3-PE-Cy5) and Natural Killer (NK) cocktail (anti-CD45-FITC/CD19-ECD/CD3-PE-Cy5/CD56-PE) (Beckman Coulter). Analysis was performed on a Cytomics FC 500 using CXP software (Beckman Coulter). Sphero Rainbow Calibration particles (Spherotech, Lake Forest, IL, USA) were used for periodic calibration of channels.

RTX and anti-RTX levels in serum

Sera were tested for RTX (PG-M-127-01-PROMONITOR®-RTX) and anti-RTX levels PG-M-127-02-PROMONITOR®-RTX) using specific ELISAs according to the manufacturer's instructions.

Table 1. Decrements of clinical and analytical values at 180 days after the first rituximab infusion

	Group A (>56,000 pg/mL)	Group B (<56,000 pg/mL)	p
▼180 DAS28	1.7±1.3	1.7±1	0.96
▼180 ESR (mm/h)			
▼180 ESR (mm/h)	9.1±9.1	37.1±8	0.038
▼180 IgG (mg/dL)	3±3.6	23.3±6.9	0.018
▼180 IgA (mg/dL)	0.8±16.3	16.3±22.6	0.092
▼180 IgM (mg/dL)	24.2±4.5	39.2±4.2	0.03
▼180 RF (UI/mL)	56.7±6.4	53.3±10.9	0.7
▼180 C3 (mg/dL)	- 13±4.7	6±3.7	0.005
▼180 C4 (mg/dL)	- 20±4.6	- 3.7±7.8	0.07
B cells on day 180 (cells/mL)	9.7±3.2	1.3±0.4	0.03

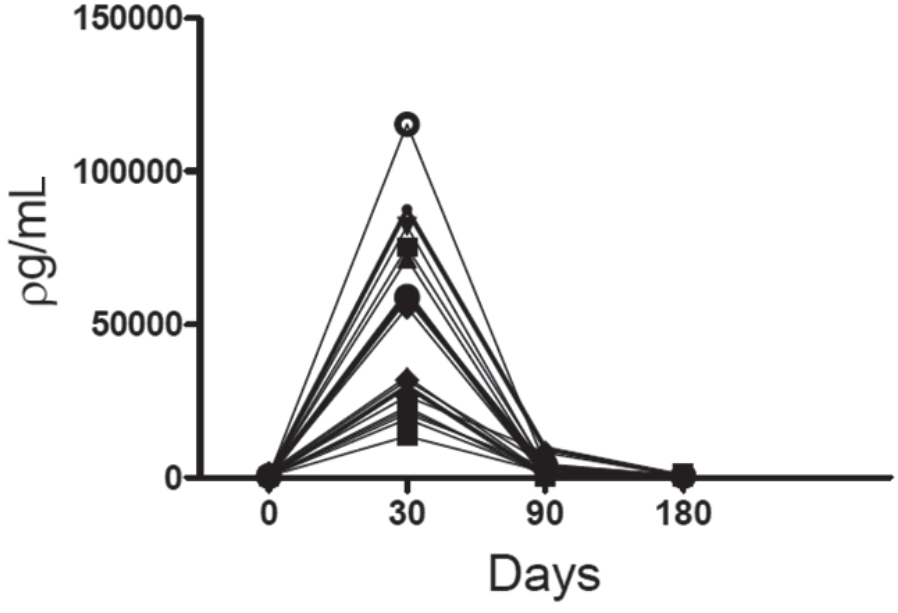

Figure 1. Rituximab levels evolution from baseline to 180 days after RTX first infusion. RTX levels were highly variable from 13,000 to 115,000 pg/mL. After the first infusion for 90 days, RTX levels were still detectable in 80% of patients

Statistical Analysis

Calculations were performed using Statistical Packages of Social Sciences version 18 (SPSS Inc.; Chicago, IL, USA). Results were expressed as mean±SEM. Mann–Whitney *U* test and Wilcoxon's test were used for independent and related variables respectively, at different time-points during the follow-up. A p-value of ≤0.05 was considered statistically significant.

Results

A total of 34 patients were included in the study. The mean age of the patients was 62.4 (37–81) years, and 93.1% were women. Disease duration was 15.7 (10.7) years. Only 9.09% were RF and ACPA negative. Of the 34 patients, 55.6% were on monotherapy, and 33.3% were on methotrexate.

RTX and anti-RTX levels

As shown in Figure 1, the peak of RTX levels was observed 30 days after initiating treatment. As in previous studies (6-9), RTX levels were highly variable and ranged from 13,000 pg/mL to 115,000 pg/mL. After treatment for 90 days, RTX levels decreased significantly but were detectable in 80% of patients. No RTX was detected on day 180. As in other studies, no anti-RTX antibodies (ARAs) were detected during the follow-up (9).

Effects of RTX levels on clinical and laboratory parameters and B cell recovery

To analyze the association between RTX levels and clinical and immunological parameters, patients were segregated into two groups according to RTX levels at 30 days. Patients with RTX levels >56,000 pg/mL (group H: 51.5% of patients) and those with RTX levels <56,000 pg/mL (group L: 48.5% of patients). Patients from both groups were comparable before treatment. As shown in Table 1, decreases in DAS28, CRP, and RF were similar in both groups at 180 days. However, group L patients presented a larger decrease of ESR, IgG, IgA, IgM, C3, and C4.

As shown in Figure 2, depletion kinetics and recovery of B cells were different in the two groups of patients. Patients from group H showed a greater initial B cell depletion than those from group L. This greater depletion was maintained up to day 90. B cell recovery was significantly faster in group H patients. By day 180, B cells were still depleted in group L (20% of group H patients and 44% of group L patients presented <1 CD19+ cell/mL) (p=0.028).

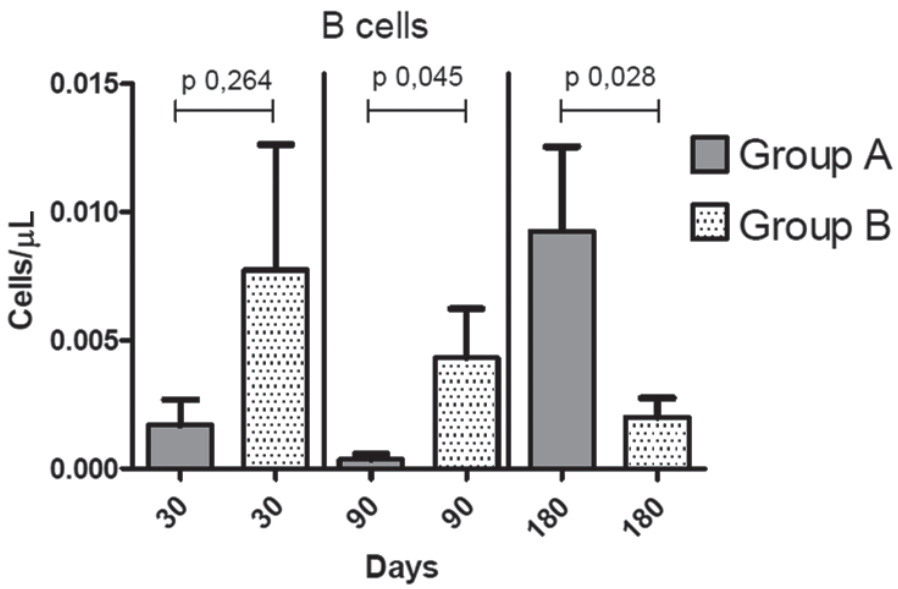

Figure 2. B cell evolution in both groups. Group with higher levels of rituximab showed earlier peripheral B cell depletion and also an earlier B cell recovery

Discussion

We found that higher decreases of immunological parameters were associated with lower levels of RTX. In addition, the recovery of B cells was delayed in patients with lower levels of RTX. This finding appears to contradict previous studies in patients with lymphoma or lupus treated with RTX that higher levels of RTX in sera were associated with better outcomes and a stronger B cell depletion (3). Differences in RTX levels and in B cell recovery pace between patients with RA and lymphoma are not surprising because doses of the drug and B cell distribution differ significantly in these two diseases. In RA, most active B lymphocytes are found in the germinal centers of the lymph nodes, bone marrow, spleen, or other similar structures, such as lymphocyte aggregates in the synovium. These were probably memory B cells searching for an environment that favors their stimulation and survival (10).

In other biological therapies, higher drug concentrations in the peripheral blood of patients with RA were related to better outcomes (4). However, few groups have studied the RTX levels and the association with clinical or radiological response and B cell depletion in patients with RA. One previous study, with results similar to our own, reported a great variability in serum levels and the highest value on day 30. The authors found that patients with ARAs had lower RTX levels. However, they did not find any association between RTX levels and clinical outcome, radiological progression, or synovial B cell depletion at 12 or 16 weeks (8). Another study observed, as we did, that higher RTX levels were associated with higher peripheral B lymphocyte depletion levels on days 30 and 90 after the first infusion (7). Unfortunately, the authors did not show results further than 90 days, the time when we observed that peripheral B cell recovery began in patients with higher levels of RTX.

There are several explanations for highly variable levels of RTX in patients with RA: (1) differences in RTX metabolism, (2) polymorphisms in Fc neonatal receptor, a molecule involved in the recycling of IgG1 (11), and (3) differences in RTX distribution in the body compartments. The complex distribution of RTX in different body compartments could also explain differences among diseases. Positron emission tomography/computed tomography imaging has shown that immediately after the infusion, RTX is located in the spleen and, in a smaller quantity, in the bone marrow. After infusion for 24 h, RTX is mostly located in the thyroid gland. It then spreads through the body in a variable manner (12-13). These findings could explain the total depletion of B cells in the spleen, but not in other compartments, in patients with non-Hodgkin lymphoma (14).

Based on these data, we speculate that RTX is predominantly distributed in the extravascular compartments in group L patients, consistent with lower RTX levels in the blood. These patients could have a poorer initial B cell depletion in the blood but a better B cell depletion in the germinal centers. Therefore, they could have a higher decrease in immunoglobulins in the peripheral blood. In addition, poorer initial depletion of B cells could induce lower levels of B cell activating factor (15), thus delaying B cell recovery in group L patients.

This hypothesis could also explain why the treatment response in patients with RA with higher levels of RTX in sera was comparable with those with lower levels of RTX. It also could suggest that patients with lower RTX levels could be candidates of RTX treatment on demand instead of every 6 months. More studies should be conducted to clarify this issue.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Hospital de la Santa Creu i Sant Pau.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - C.D.T., C.D.L., J.C., S.V.; Design - C.D.T., M.A.O., M.S., C.D.L., S.V.; Supervision - C.D.L., J.C., S.V.; Data Collection and/or Processing - C.D.T., M.A.O., M.S., H.C., S.V.; Analysis and/or Interpretation - C.D.T., M.A.O., C.D.L., S.V.; Writing Manuscript - C.D.T., M.A.O., S.V.; Critical Review - C.D.T., M.A.O., M.S., H.C., C.D.L., J.C., S.V.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Leandro MJ, Cooper N, Cambridge G, Ehrenstein MR, Edwards JC. Bone marrow B-lineage cells in patients with rheumatoid arthritis following rituximab therapy. Rheumatology (Oxford) 2007; 46: 29-36. [CrossRef]
2. Thurlings RM, Vos K, Wijbrandts CA, Zwinderman AH, Gerlag DM, Tak PP. Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response. Ann Rheum Dis 2008; 67: 917-25. [CrossRef]
3. Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997; 90: 2188-95.
4. Jamnitski A, Krieckaert CL, Nurmohamed MT, Hart MH, Dijkmans BA, Aarden L, et al. Patients non-responding to etanercept obtain lower etanercept concentrations compared with responding patients. Ann Rheum Dis 2012; 71: 88-91. [CrossRef]
5. Diaz-Torne C, Ortiz de Juana MA, Geli C, Canto E, Laiz A, Corominas H, et al. Rituximab-induced interleukin-15 reduction associated with clinical improvement in rheumatoid arthritis. Immunology 2014; 142: 354-62. [CrossRef]
6. Boumans M, Teng O, Thurlings R, Bijlsma J, Gerlag D, Huizinga T, et al. Progression of structural damage is not related to rituximab serum levels in rheumatoid arthritis patients. Rheumatology (Oxford) 2013; 52: 1462-6. [CrossRef]

7. Reddy V, Croca S, Gerona D, De La Torre I, Isenberg D, McDonald V, et al. Serum rituximab levels and efficiency of B cell depletion: differences between patients with rheumatoid arthritis and systemic lupus erythematosus. Rheumatology (Oxford) 2013; 52: 951-2. [CrossRef]
8. Thurlings RM, Teng O, Vos K, Gerlag DM, Aarden L, Stapel SO, et al. Clinical response, pharmacokinetics, development of human anti-chimaeric antibodies, and synovial tissue response to rituximab treatment in patients with rheumatoid arthritis. Ann Rheum Dis 2010; 69: 409-12. [CrossRef]
9. Mazilu D, Opris D, Gainaru C, Iliuta M, Apetrei N, Luca G, et al. Monitoring drug and antidrug levels: a rational approach in rheumatoid arthritis patients treated with biologic agents who experience inadequate response while being on a stable biologic treatment. Biomed Res Int 2014; 2014: 702701. [CrossRef]
10. Silverman GJ, Boyle DL. Understanding the mechanistic basis in rheumatoid arthritis for clinical response to anti-CD20 therapy: the B-cell roadblock hypothesis. Immunol Rev 2008; 223: 175-85. [CrossRef]
11. Challa DK, Velmurugan R, Ober RJ, Sally Ward E. FcRn: from molecular interactions to regulation of IgG pharmacokinetics and functions. Curr Top Microbiol Immunol 2014; 382: 249-72. [CrossRef]
12. Tran L, Huitema AD, van Rijswijk MH, Dinant HJ, Baars JW, Beijnen JH, et al. CD20 antigen imaging with (1)(2)(4)I-rituximab PET/CT in patients with rheumatoid arthritis. Hum Antibodies 2011; 20: 29-35. [CrossRef]
13. Tran L, Vogel WV, Sinaasappel M, Muller S, Baars JW, van Rijswijk M, et al. The pharmacokinetics of (1)(2)(4)I-rituximab in patients with rheumatoid arthritis. Hum Antibodies 2011; 20: 7-14. [CrossRef]
14. Cioc AM, Vanderwerf SM, Peterson BA, Robu VG, Forster CL, Pambuccian SE. Rituximab-induced changes in hematolymphoid tissues found at autopsy. Am J Clin Pathol 2008; 130: 604-12. [CrossRef]
15. Cambridge G, Perry HC, Nogueira L, Serre G, Parsons HM, De La Torre I, et al. The effect of B-cell depletion therapy on serological evidence of B-cell and plasmablast activation in patients with rheumatoid arthritis over multiple cycles of rituximab treatment. J Autoimmun 2014; 50: 67-76. [CrossRef]