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

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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

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.

### 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.

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

| Parameter         | 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)   | 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 |

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).
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 declared that this study has received no financial support.

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

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