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A longitudinal analysis of SLE patients treated with rituximab (anti-CD20): Factors associated with B lymphocyte recovery ☆

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Abstract Identifying factors associated with B lymphocyte depletion and recovery may aid the development of individualized treatment regimens, optimizing therapy for patients with autoimmune disease. In this study, 12 patients with active SLE were monitored at baseline and monthly following treatment with rituximab. The number and phenotype of peripheral blood B lymphocytes, T lymphocytes and natural killer cells were correlated with the extent and longevity of B lymphocyte depletion. This analysis generated three candidate biomarkers for lymphocyte monitoring in patients with autoimmune disease who are treated with rituximab: circulating transitional B cells, the κ:λ ratio and natural killer cells. Further refinement of these potential biomarkers may lead to a better understanding of the role of B cells in disease pathogenesis and a more rational use of B cell depletion therapies.

Introduction

Rituximab, a chimeric anti-CD20 monoclonal antibody, was first approved in 1997 as an effective treatment for a subpopulation of CD20$^+$ B cell non-Hodgkin's lymphoma [1,2]. It is now also being used to treat autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus in which B cells are thought to play a pathogenic role. It has been hypothesized that elimination of pre-plasma B cells in patients with a preferential recurrence of naive cells will reestablish B cell tolerance during reconstitution [3–5].

Abbreviation: SLE, systemic lupus erythematosus.

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Recent studies have described the pattern of depletion and reconstitution of the circulating B cell compartment following the treatment of patients with both rheumatoid arthritis and SLE with rituximab [6–10]. In patients with rheumatoid arthritis, depletion of B lymphocytes occurs uniformly and regeneration follows a characteristic pattern with an early presence of immature cells in addition to recirculating plasma cells [6]. The repopulation of the memory B cell compartment is delayed in these patients. In contrast, B cell depletion is highly variable among SLE patients ranging from complete and prolonged to transient to incomplete with an increase in the percentage of naive B cells after reconstitution [9].

Given the documented variability in the biological response to B-cell-targeted therapy in SLE patients, the current study employed a longitudinal analysis of B, T and NK lymphocytes in individual patients to gain further insight into the kinetics of B cell depletion and the factors associated with subsequent auto-reconstitution. Findings included (1) three out of four patients with a high proportion of circulating transitional B lymphocytes at baseline exhibited short-lived B cell depletion, suggesting that the baseline phenotype may predict the length of depletion in some patients. (2) Transitional B lymphocytes were usually the first cells to return, irrespective of the baseline phenotype. (3) A variable kappa:lambda light chain ratio (κ:λ) was observed when the B cells first returned. (4) A relative and absolute increase of circulating natural killer cells was observed during B lymphocyte depletion in 8 out of 12 patients.

Materials and methods

Patients/Clinical parameters

Patients, aged 23–56 years, who fulfilled the American College of Rheumatology criteria for SLE [11] were recruited from the University of Pennsylvania to receive rituximab as part of a multi-center phase 1, open label trial aimed to assess the safety and efficacy of rituximab in the management of SLE. All patients had SLE for at least 6 months prior to the start of the study, had failed a least one immunosuppressive drug and had active disease. Each patient underwent a 1-month washout period devoid of any immunosuppressive medications except for prednisone. Exclusion criteria included severe end-organ disease, a history of malignancy, HIV, alcohol or drug abuse, active infections and pregnancy or lactation. Detailed informed consent was obtained from all patients. These studies were performed in accordance with a protocol that was approved by the University of Pennsylvania Institutional Review Board. The present communication describes data for 12 SLE patients and 13 control subjects recruited at the University of Pennsylvania for whom we obtained detailed flow cytometric data.

Rituximab was provided by Genentech (San Francisco, CA). Ten patients received a full course with the dose approved for use in lymphoma (375 mg/m^2 intravenously weekly for 4 weeks). The patients were pretreated with 50 mg of oral diphenhydramine, 650 mg of oral acetaminophen, and 100 mg of intravenous methylprednisolone 30 min before each infusion. Two patients received partial doses due to the development of serum sickness (patient 20) and voluntary withdrawal (patient 24). One patient was re-treated with rituximab after week 31 of the study (patient 22). The clinical response was assessed primarily by D.A.A., calculating a SLEDAI score at baseline and at weeks 4, 7, 11, 15, 19, 27, 39, and 55.

Peripheral blood lymphocyte immunophenotyping

Flow cytometric analysis of T and B lymphocytes and natural killer (NK) cells was performed at baseline for each patient and at 4- to 8-week intervals following rituximab therapy for an average of 54 weeks (range 41–60). Flow cytometry was not performed at the following time points: subject 4 (5 months), subject 5 (6 months), subject 7 (4 and 10 months), subject 9 (3 and 12 months), subject 15 (4 months), subject 22 (3 and 10 months), subject 23 (10 months) and subject 24 (3, 5, 10, 11 and 12 months; this subject withdrew from the study.) A similar analysis was performed at a single time point for 13 apparently healthy normal controls. Monoclonal antibodies were added directly to 150 μl aliquots of fresh whole blood (anticoagulated in sodium EDTA) and incubated at room temperature for 20 min. Red blood cells were lysed in ammonium chloride (BD Pharm Lyse) and the remaining mononuclear cells were washed in Dulbecco's PBS with 5% fetal calf serum and sodium azide. Cells were fixed in 1% paraformaldehyde (Electron Microscopy Sciences). Only fresh whole blood specimens (<24 h old) were analyzed in this study. A seven-tube, four-color panel with antibodies against the following cell surface markers (clone) was used: CD3 (HIT3a), CD4 (SK3), CD8 (SK1), CD19 (SJ25C1 or HIB19), CD27 (MT271), CD38 (HIT2), IgM (G20–127), IgD (IA6-2), kappa (G20–193), lambda (JDC-12), CD56 (B159) and CD16 (clone 3G8) (BD Pharmingen San Jose, CA). Analysis was performed on a FACSCalibur flow cytometer with CellQuest software (Version 5.2.1, Becton Dickinson, San Jose, CA). After gating on lymphocytes utilizing forward scatter and side scatter, T cells were identified based on CD3 expression and analyzed for CD4 and CD8 expression. B cells were identified based on CD19 expression and analyzed for CD27, CD38, IgM, IgD, kappa and lambda expression. NK cells were identified as CD3-negative, CD56 and/or CD16 positive lymphocytes. Negative populations were determined using anti-mouse isotype controls. We collected either 10,000 CD3$^+$ or CD19$^+$ events per tube or the entire sample if this number of CD19$^+$ cells was not reached. Absolute cell numbers were calculated using the white blood cell count and the percentage of lymphocytes from a complete blood count (CBC) usually obtained on the same day (or at most within 1 week) of the immunophenotyping sample.

Statistical analysis

Mean values, standard deviation and standard error of the mean were calculated for clinically significant groups. Statistical significance between patients and controls was determined using a two-sided Student's *t*-test with a *p* value of <0.05, with the caveat that the subject size is small and that small differences between groups will be missed in this analysis. Correlation coefficients using ranked and unranked