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Rituximab Therapy for Primary Sjögren's Syndrome: An Open-Label Clinical Trial and Mechanistic Analysis

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Abstract

Objective—To study the safety and clinical efficacy of rituximab therapy for primary Sjögren's syndrome, as well as investigate its mechanisms.

Methods—Patients with primary Sjögren's syndrome were enrolled in an open-label trial and received rituximab (1 g) on days 1 and 15 and followed through week 52. The primary endpoint was safety, with secondary endpoints evaluating clinical and biologic efficacy. Blood was obtained for enumeration of lymphocyte subsets, measurement of serum autoantibodies and BAFF levels, and analysis of gene expression.

Results—Twelve female subjects with primary Sjögren's syndrome were administered rituximab. They had a median (range) age of 51 (34–69) years and a median (range) disease duration of 8.0 (2–18) years. We observed no unexpected toxicities from rituximab therapy. Modest improvements were observed at week 26 in patient-reported symptoms of fatigue and oral dryness, with no significant improvement in the objective measures of lacrimal and salivary gland function. The recovery of blood B cells following the nadir from rituximab therapy was characterized by a predominance of transitional B cells and a lack of memory B cells. While blood B cell depletion was associated with an increase in serum BAFF levels, no significant changes were observed in the levels of serum anti-Ro/SSA, anti-La/SSB, and anti-muscarinic receptor 3 autoantibodies or in the blood IFN signature.

Conclusion—In primary Sjögren's syndrome, a single treatment course of rituximab was not associated with any unexpected toxicities and led to only modest clinical benefits despite effective depletion of blood B cells.

Primary Sjögren's syndrome is among the most common of the connective tissue diseases. For women, its prevalence in the United Kingdom has been estimated to be 0.1 – 0.6 % (1). The disease is characterized by the presence of keratoconjunctivitis sicca (dry eyes),

xerostomia (dry mouth), serum antinuclear antibodies, and chronic salivary gland inflammation, as well as the occurrence of systemic features, such as profound fatigue, polyarthralgia/polyarthritis, interstitial lung disease, peripheral neuropathy, and leukocytoclastic vasculitis (2, 3). Patients with primary Sjögren's syndrome are also at increased risk of developing B cell lymphoma (4).

The treatment of primary Sjögren's syndrome is largely based on alleviation of symptoms and includes the use of topical cyclosporine for management of dry eyes, sialogogues (oral muscarinic agonists), hydroxychloroquine, and low doses of prednisone (5). Patients with more serious systemic manifestations may require more intensive therapy with glucocorticoids and other immunosuppressive agents. However, no drugs have been shown in well-designed clinical trials of patients with primary Sjögren's syndrome to reduce disease activity or prevent damage.

The potential clinical utility of rituximab therapy has been recently investigated in primary Sjögren's syndrome (6–10) owing to its proven efficacy in other chronic inflammatory diseases such as rheumatoid arthritis (11, 12) and systemic vasculitis (13) and its effects on potential disease-inciting B cells. The importance of abnormal B cell responses in the mechanisms of primary Sjögren's syndrome is strongly suggested by the presence of serum autoantibodies, most notably anti-Ro/SSA and anti-La/SSB antibodies (3). The benign and malignant B cell monoclonal proliferations in the blood and salivary gland tissue of patients with primary Sjögren's syndrome (14), as well as the abnormalities in B cell memory (15, 16), provide further evidence that B cells play an important role in the pathophysiology of this condition. We therefore conducted an open-label study of rituximab, a potent B cell depleter, to evaluate the safety and possible clinical efficacy of this approach in primary Sjögren's syndrome, as well as determine its effects on blood B cell subsets, autoantibodies, cytokines, and gene transcripts.

Patients and Methods

Study design and treatment

The study was a prospective, open-label, single arm, phase I study of rituximab therapy for patients with primary Sjögren's syndrome (ClinicalTrials.gov. identifier NCT0012101829). Twelve subjects received two 1,000 mg infusions of rituximab two weeks apart using a standard protocol with escalation of the infusion rate to a maximum of 400 mg/hour. All subjects were pre-treated with 50 mg of oral diphenhydramine, 650 mg of oral acetaminophen, and 100 mg of intravenous methylprednisolone approximately 30 minutes before each of the infusions. The patients returned for follow-up visits at weeks 4, 8, 14, 26, 30, 36, and 52. Diphtheria and tetanus toxoid (Td) and a pneumococcal polyvalent-23 vaccine were administered at week 26 to 8 of the subjects for assessment of immunocompetence.

Study oversight

The study was approved by the institutional review boards (IRBs) at Duke University Medical Center and the University of Pennsylvania. All subjects provided informed consent. The study was designed by the investigators and coordinated by the National Institutes of Allergy and Infectious Disease, the study sponsor, and Rho, Inc., which managed the collection and quality control of the data and performed the statistical analyses. Rituximab was provided at no cost by Genentech, which had no other role in the design, conduct, or analysis of the study.

Patient population

Eligible patients were adults between the ages of 18 and 75 years that met classification criteria for primary Sjögren's syndrome as defined by the American-European Consensus Group (17). In addition, patients had one or more of the following systemic manifestations: fatigue (> 50 mm on a 100 mm visual analogue scale [VAS]); joint pain (> 50 mm on a 100 mm VAS); severe parotid gland swelling; peripheral neuropathy; interstitial lung disease; leukocytoclastic vasculitis; interstitial nephritis; or other extraglandular disease causing organ system dysfunction. Subjects of reproductive potential agreed to use an acceptable method of birth control during treatment and for 12 months following treatment.

Subjects were allowed concomitant therapy with nonsteroidal anti-inflammatory drugs, cevimeline, pilocarpine, ophthalmic cyclosporine, and hydroxychloroquine, provided they were maintained at the baseline doses. Concurrent therapy with prednisone (≤ 10 mg/d) was permitted if doses had been stable for at least 2 weeks prior to study entry and were kept constant during the study. Subjects were excluded from the study if they had previously been treated with rituximab or if they had been recently treated with the following medications: cyclophosphamide within 24 weeks; methotrexate, azathioprine, cyclosporine or mycophenolate mofetil within 4 weeks; etanercept within 4 weeks; adalimumab within 8 weeks; or infliximab within 12 weeks. Subjects taking potent anticholinergic agents, such as tricyclic antidepressants, antihistamines, phenothiazine, and antiparkinsonian drugs were not allowed to participate in the study. Patients were excluded if they had active infection; chronic or persistent infection that might be worsened by immunosuppressive therapy (e.g. human immunodeficiency virus, hepatitis B or C, tuberculosis); known coronary artery disease or a history of significant cardiac arrhythmias or severe congestive heart failure; pregnancy; ongoing oral anticoagulant therapy; a history of alcohol or substance abuse; prior head and neck radiation therapy; history of sarcoidosis; history of a positive PPD without documentation of treatment for active or latent tuberculosis; history of severe pulmonary disease (FVC < 50% predicted, DLCO < 50% predicted; resting oxygen saturation < 95%); history of malignancy, except for resected basal cell or squamous cell carcinoma of the skin, cervical dysplasia, or in situ cervical cancer grade 1 within the last 5 years; abnormal laboratory results (absolute neutrophil count < 1000/mm³, platelets < 100,000/mm³, hemoglobin < 9 g/dl, serum creatinine ≥ 2.0 mg/dl, aspartate aminotransferase or alanine aminotransferase > 2 times the upper limit of normal); or administration of a live vaccine within the past 3 months.

Study endpoints

The primary safety endpoint was the proportion of patients experiencing a grade 3, grade 4, or grade 5 adverse event (AE) (National Cancer Institute-Common Terminology Criteria for Adverse Events) judged by the investigator to be possibly, probably, or definitely related to rituximab therapy. The other goal of the study was to obtain preliminary evidence of clinical and biologic activity using measures of exocrine gland function and other disease features, as well as immune system function. For the clinical and biological endpoints, we focused our analysis on the changes between weeks 0 and 26.

Clinical assessments

Safety was evaluated at each visit by monitoring for AEs, including changes in routine laboratory parameters. Assessments of clinical efficacy were performed at weeks 8, 14, 26, 36, and 52 and included a Sjögren's Syndrome Symptom Survey (18), physician and patient global assessment (100 mm visual analog scales), unanesthetized Schirmer's test, slit lamp examination with lissamine green staining and measurement of unstimulated and stimulated whole salivary flow rate. For the assessment of salivary flow, the subjects were instructed to hold the morning dose of secretagogue and eat nothing by mouth for at least 60 minutes

prior to measurement of salivary flow. Unstimulated saliva flow was determined by having the subject expectorate into a pre-weighed 50 cm³ centrifuge tube for 15 minutes. Saliva samples were weighed on an analytical balance to quantify the volume over the 15-minute collection period (1 gm = 1 mL). Subsequently, subjects were treated with 5 mg of oral pilocarpine; 60 minutes later they had assessment of the stimulated salivary flow rate by collection of another 15 minute sample. In addition, quality of life was examined at weeks 0 and 26 using the SF-36 questionnaire (19).

Flow cytometry

Venous blood that was less than 24 hours old was processed for flow cytometry as described previously (20, 21). After washing and Fc blockade, cells were stained with the following antibody fluorochrome combinations in multiple 4-color combinations (all antibodies were purchased from BD Biosciences, San Jose, CA): CD27-FITC (M-7271), CD3-FITC (SK7), CD38-PE (HIT2), CD8-PE (HIT8a), CD16-PE (3G8), CD56-PE (B159), CD20-PerCPCy5.5 (L27), CD4-PerCP (SK3), CD8-PerCP (SK1), CD19-APC (HIB19), CD45-APC (H130), CD14-APC (M5E2). After staining and lysis of red blood cells, the white blood cell pellets were washed and fixed in paraformaldehyde. Data from stained cells were acquired on a FACS Calibur instrument (BD Biosciences) and analyzed using CellQuest Pro software version 5.2 (BD Biosciences). Approximately 10,000 CD19⁺ lymphocytes were analyzed per tube or, in samples with low numbers of B cells, the maximum number of events was acquired by draining the tube. For comparison with the subjects in the clinical trial, blood was obtained with IRB approval at the University of Pennsylvania from 15 controls (11 females, 4 males), with a median $\pm$ IQR age of 39.5 (27, 49). The control subjects were not part of the current trial and younger than the subjects enrolled in the study herein.

Measurement of autoantibodies

Serum IgM rheumatoid factor was determined by nephelometry. Serum antibodies to SS-A (Ro) and SS-B (La) were measured by ELISA in the Clinical Immunology Laboratory at the Hospital of the University of Pennsylvania. For assay of anti-muscarinic receptor 3 (M3R) antibodies, patient sera were incubated with Flp-In Chinese Hamster Ovary (CHO) cells transfected with a human M3R muscarinic receptor construct as previously described (22). Transfected or non-transfected control cells were incubated with 5 μ l of serum for two hours on ice, washed once, and stained with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibodies to human IgG or IgA. After washing, cells were analyzed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). The results are expressed as mean fluorescence intensity (MFI).

Serum BAFF levels

ELISA was performed using anti-hBAFF mAb clone B4H7.2 for coating and biotin-labeled anti-hBAFF clone A9C9.1 for detection. Standard curves were constructed with recombinant BAFF (all Biogen Idec, Cambridge, MA, USA). To control for inter-assay variations in each freshly prepared standard curve, a two-point inter-assay control standard was applied. Serum was diluted 1:10 (or higher if necessary for high BAFF levels) and tested in triplicate.

Gene expression profiling

Peripheral blood was collected into two PAXgene RNA tubes (PreAnalytiX, Switzerland) and total RNA was extracted using PAXgene 96 Blood RNA Kits (Qiagen, Valencia, California). Excess globin transcripts were removed using Ambion GLOBINclear (Life Technologies, Grand Island, New York), following manufacturer's protocols. RNA concentrations and quality were assessed using an Agilent 2100 Bioanalyzer (quality

threshold 28s:18srRNA ratios above 1.0; RNA concentration >70 ng/ul and no more than 14 ul for optimum cleaning). Biotinylated, amplified RNA was produced from 300 ng RNA using a modification of the Eberwine protocol (23) as described in the Illumina® TotalPrep RNA Amplification Kit (Ambion). The cRNA was hybridized overnight at 58°C to HumanWG-6 Expression BeadChip™ microarrays (Illumina Corp., San Diego), washed under high stringency conditions, labeled with streptavidin-Cy3, and scanned. Raw intensity values were background subtracted using Illumina BeadStudio software. Probe level analysis was performed using the BeadConductor Lumi package for R; a package specifically written to process Illumina microarray data (www.bioconductor.org). The raw gene expression data were subjected to variance-stabilizing transformation; robust splines were then applied for normalization. The eight samples with data for each time point (Baseline, post-8 week, post-26 week, post-52 week) were included in the analysis. The expression data were not normalized for the numbers of B cells in the blood. Paired t-tests and p values were calculated at each 8-week, 26-week and 52-week time point compared to baseline. IPA 9.0 software (Ingenuity Pathway Analysis, Ingenuity Systems, Redwood City, California) was utilized to map transcripts to canonical pathways.

Statistical analysis

The sample size of 12 patients was chosen to obtain sufficient information about safety in this disease population, with a secondary objective of obtaining preliminary information about its possible clinical and biological activity. A sample size of 12 subjects is the minimum size required to generate a one-sided 90 % confidence interval that excludes a prevalence of 30 % for an event if no more than one treatment-related AE of unacceptable severity is observed. The frequencies of different B cell subsets were compared between patients with primary Sjögren's syndrome and healthy controls using a two-tailed exact Mann Whitney test. P-values for all other tests were based on the Wilcoxon Signed-rank test and examined the null hypothesis that the median difference score between the baseline visit and the Week 26 visit is equal to 0. A p-value of 0.05 or less was considered to be statistically significant. No adjustments were made for multiple comparisons. Calculations were performed using SAS version 9.1 software, or higher (SAS Institute, Cary, NC).

Results

Recruitment took place between April, 2005 and July, 2006, with six subjects enrolled each from Duke University Medical Center and the University of Pennsylvania. All 12 subjects received the full dose of rituximab and completed study follow-up through week 52, and were included in the safety and efficacy analyses. Their baseline characteristics are shown in Table 1. Most of the enrolled participants had relatively low baseline rates of unstimulated and stimulated whole salivary flow. Eight subjects were taking hydroxychloroquine therapy, while only 3 subjects were receiving oral prednisone 10 mg/d. Four of the patients were receiving an oral secretagogue (pilocarpine, n = 2; cevimeline, n = 2).

Safety

The rituximab infusions were generally well-tolerated in this study (Table 2, Supplementary Table 1). Two subjects suffered a serious adverse event (SAE), including 1 patient that had a grade 2 reaction to a pneumococcal vaccine consisting of local (pain, swelling, and numbness) and systemic (fever, myalgia) symptoms that resulted in an emergency department evaluation and treatment with parenteral and oral antibiotics. Of note, another patient in the study had a grade 2 vaccination reaction and an additional patient had a grade 2 vaccination reaction associated with fever and chills; neither of these AEs were SAEs. None of the other 5 subjects who received both a pneumococcal and a diphtheria and tetanus toxoid vaccine had AEs from these immunizations. In all 3 cases, these AEs resolved

without apparent sequelae. No subsequent vaccinations were administered following these AEs owing to the unanticipated severity of these reactions. The other SAE was a squamous cell carcinoma of the skin that occurred 301 days after administration of rituximab that was considered to be possibly related to the study drug. We did not observe any serum sickness-like reactions in this study.

Clinical efficacy

The results showed significant but modest levels of improvement between week 0 and 26 in the both the physician's (median decrease = 26 mm; $p = 0.012$) and patient's (median decrease = 8.5 mm; $p = 0.009$) global rating of disease activity. Although positive trends towards subjective improvement in dryness were observed in many items of the survey, only the changes in the ratings of tongue dryness (median decrease = 11.1 mm; $p = 0.007$), level of thirst (median decrease = 35.5 mm; $p = 0.005$), level of oral discomfort (median decrease = 23.5 mm; $p = 0.02$), and level of overall fatigue (median decrease = 18.4 mm; $p = 0.042$) reached statistical significance. There was no significant improvement in joint pain (median decrease = 4.0 mm; $p = 0.077$). We also did not find any statistically significant improvement between week 0 and 26 in the unstimulated (median change = 0.01; $p = 0.287$) or stimulated (median change = 0.005; $p = 0.718$) whole salivary flow. There were also no significant changes in tear production, as measured by the unanesthetized Schirmer's test, or ocular surface dryness, as determined by a modified von Bijsterveld scoring system (grade 0–18). Although no significant improvement was observed between week 0 and 26 in the summary measures of the SF-36 for physical and mental functioning, a statistically significant increase was found during this period in the scores on the vitality scale ($p = 0.006$).

Peripheral blood B cell depletion and reconstitution

The absolute peripheral blood CD19⁺ lymphocyte (B cell) counts at baseline ranged from 45–341 cells/ μ l (median=178 cells/ μ l). Following rituximab treatment, all of the 12 subjects by week 8 or 14 showed greater than 95% depletion of blood B cells (Figure 1). The blood B cell counts had a nadir at weeks 8 and 14 and began to rise by week 26; they returned to 62% or greater of baseline values by week 52 in 8 of 12 subjects.

The subsets of circulating B cells were analyzed according to the surface expression of CD38 and CD27, a previously validated approach (21, 24). Using these markers, we defined six different subsets: transitional B cells (CD38⁺⁺, CD27⁻), mature naïve B cells (CD38⁺, CD27⁻), mature activated memory B cells (CD38⁺, CD27⁺), resting memory B cells (CD38⁻, CD27⁺), plasmablasts (CD38⁺⁺, CD27⁺⁺), and so-called double-negative B cells (CD38⁻, CD27⁻). Compared to our healthy controls, the patients with primary Sjögren's syndrome at baseline had a greater number of transitional B cells and a lower number of memory B cells (Supplementary Figure 1). Therefore, the distribution of circulating B cell subsets in the 12 patients from our study was similar to that of patients with primary Sjögren's syndrome reported previously (10, 15).

After rituximab therapy, the initial wave of repopulating blood B cells was comprised mainly of transitional B cells (Figure 2, Table 3). At week 26, the median number of transitional B cells (CD38⁺⁺, CD27⁻) in the circulation was 10.3cells/ μ l (65% of total CD19⁺ B cells). By comparison, the naïve mature B cells at week 26 comprised on average only 8.3% (median = 0.57cells/ μ l) of the total CD19⁺ B cells. Mature activated and resting memory B cells (CD38⁺, CD27⁺; and CD38⁻/CD27⁺) were relatively rare during this initial phase of reconstitution, comprising approximately 4% (median) of the total circulating CD19⁺ B cells. By week 52, the median number of circulating transitional B cells had increased to 21.3 cells/ μ l (19.3% of total CD19⁺ B cells). While at the same time, the median number of mature naïve B cells (CD38⁺, CD27⁻) had increased to 75.9 cells/ μ l (73.1% of

total CD19⁺ B cells). The median number of circulating mature activated (CD38⁺, CD27⁺) and resting memory (CD38⁻, CD27⁺) B cells remained diminished both in absolute and relative terms at week 52, representing only 3.2% and 0.4% of the total CD19⁺ B cells. Thus, 52 weeks following rituximab therapy, the more mature B cell subsets had not yet fully repopulated the circulating pool.

Analysis of blood T and NK cells, and monocytes

Rituximab therapy was not associated with any substantial changes in the number or percentage of blood CD3⁺, CD4⁺, and CD8⁺ T cells, CD16⁺CD56⁺ NK cells, and CD14⁺ or CD11b monocytes (data not shown).

Measurement of autoantibodies

Rituximab therapy had little effect on the serum levels of anti-Ro/SSA and anti-La/SSB antibodies (data not shown). However, there was a trend towards a decrease in the levels of serum rheumatoid factor, but this difference failed to reach statistical significance (median [25th, 75th percentile] week 0 = 150.0 U/L [20, 1930] vs. median [25th, 75th percentile] week 26 = 87 U/L [20, 1560]; $p = 0.109$).

Serum antibodies to the M3R have been reported to occur in primary Sjögren's syndrome and may be associated with impaired cholinergic transmission (25, 26). Baseline data for serum anti-M3R antibodies were available for 11 of the 12 subjects in the study. In general, we observed no significant changes in the serum levels of anti-MR3 antibodies between baseline and week 26, except for a lone subject whose MFI decreased from 35 to 17. Of note, this particular subject did not show an increase in either unstimulated (baseline, 0.12 ml/min; week 26, 0.06 ml/min) or stimulated (baseline, 0.33 ml/min; week 26, 0.36 ml/min) whole salivary flow rate over this time period.

Serum BAFF levels

BAFF is a key survival factor for B cells, and is important for the maintenance of peripheral B cell homeostasis (27). Serum BAFF levels are elevated in primary Sjögren's syndrome compared with healthy controls (28) and have been shown to increase after B cell depletion therapy and then gradually return to baseline following reconstitution of the circulating pool (29, 30). We found in our study that median serum BAFF levels followed a similar kinetics, rising substantially while circulating CD19⁺ B cells were maximally depleted and then returning towards baseline with reconstitution of the circulating B cell pool (Figure 3A and 3B).

Effect of rituximab therapy on the interferon signature and expression of other gene transcripts

In 8 subjects, we analyzed the effects of rituximab therapy on interferon (IFN) signature transcripts, which are up-regulated in primary Sjögren's syndrome (31), as well as the expression of other gene transcripts by comparing the transcript levels at baseline with those at weeks 8, 26, and 52 (32). Overall, we found a significant change in the expression of 94, 77, and 342 genes at these time points, respectively, compared to baseline ($p < 0.001$). Among 63 IFN-related transcripts (interferons, interferon regulatory factors, and interferon inducible genes), only IRF4, IRF8, IFITM1, IFI30, IFITM4P showed statistically significant changes ($p < 0.01$) between baseline and any of these 3 subsequent time points.

We observed a significant decrease after rituximab therapy in the expression of several B-cell related genes, including CD79A, LOC652493 (Ig kappa chain V-I region HK102-like), IGKV3D-20 (Ig kappa variable 3D-20), FCRLA, LOC647450 (similar to Ig kappa chain V-I region HK101 precursor), LOC652775 (similar to Ig kappa chain V-V region L7 precursor),

VPREB3, and BLK (see Supplementary Figure 2). Since the expression data were not normalized for the numbers of blood B cells, these changes probably reflect the depleting effects of rituximab. These changes were most pronounced at week 8 and returned towards baseline values by week 52. We also performed a pathway analysis, with the top 3 canonical pathways mapping to primary immunodeficiency signaling (baseline vs. week 8; $p < 0.01$), altered T cell and B cell signaling (baseline vs. week 8 and 26; $p < 0.01$), PI3K signaling in B lymphocytes (baseline vs. week 26; $p < 0.01$) and B cell development (baseline vs. week 8 and 26; $p < 0.01$).

Discussion

Our results show that two 1 g infusions of rituximab given 2 weeks apart produce effective depletion of circulating B cells in patients with primary Sjögren's syndrome, with a kinetics and pattern of B cell subset reconstitution similar to that observed in another study of patients with the same disease (10). The corresponding increases in serum BAFF levels following the depletion of blood B cells confirm earlier observations (32). The exploratory analysis of gene transcripts and pathways provides further evidence that rituximab therapy substantially alters B cell responses in this patient population.

Importantly, we did not detect any unexpected safety signals, except for the possibility of exaggerated vaccine reactions. Three of the subjects in our trial had an unusually severe reaction to the pneumococcal vaccine given at week 26. However, these vaccines were administered to 68 patients with rheumatoid arthritis that were treated with rituximab in a randomized, open-label trial without apparent untoward effects beyond the usual occurrence of itching, rash, and soreness at the injection site, and malaise (33). Serum sickness has occurred in some patients with primary Sjögren's syndrome following a rituximab infusion (6, 7, 9), but such an event was not observed in the current trial. We attempted to minimize this risk by pre-medicating patients with 100 mg of methylprednisolone in addition to diphenhydramine and acetaminophen.

Despite the effective depletion of blood B cells, rituximab therapy was not associated with striking clinical benefits in the current trial. Any improvements observed in an open-label study must be interpreted with caution because of the inherent subjectivity of many of the disease measures and the possibility of observer bias. Previous studies have investigated the potential clinical efficacy and safety of rituximab therapy for primary Sjögren's syndrome (6–9), but they too are limited by their small sample size, open-label design in some cases, and the lack of standardized treatment outcomes. These results are consistent with those reported in an abstract describing the results of a 122-patient, randomized, placebo-controlled study of rituximab therapy in primary Sjögren's syndrome (34). In this study, rituximab therapy was not significantly more effective than placebo in improving by 30 mm or more at least two of the four 100 mm VAS scales evaluating dryness, pain, fatigue, and global disease activity. Since relatively few subjects in our study had extraglandular manifestations beyond constitutional symptoms and joint pain, we were unable to explore the effects of rituximab therapy on severe systemic features. However, rituximab therapy has been shown in some cases to benefit such systemic features as refractory pulmonary disease, synovitis, and mixed cryoglobulinemia (35). To date, rituximab treatment has not been consistently associated with an increase in lacrimal and salivary gland function. It has been suggested that patients whose lacrimal and salivary glands have minimal secretory capacity due to long-standing disease may be particularly refractory to disease-modifying treatment (6). Indeed, 7 of the 12 subjects in our trial had stimulated salivary flow rates < 0.1 mL/min at entry and it may be argued that their glandular function had limited potential for improvement.

Rituximab therapy did not appear to affect the serum levels of anti-SSA/Ro and anti-SSB/La antibodies, although serum rheumatoid factors trended lower, as has been seen in earlier studies (8, 9). Rituximab treatment also had no impact on the serum levels of anti-M3R antibodies. This result is of interest because of the possible role of these autoantibodies in the mechanisms of impaired salivary flow. We also hypothesized that rituximab treatment would alter the blood IFN signature in primary Sjögren's syndrome based on previous studies that it can induce type 1 IFN activity in patients with rheumatoid arthritis (36). However, we were unable to demonstrate such an effect in our study, which is not unexpected based on the established relationship between type 1 IFN and BAFF. Elevated serum BAFF levels, which result from B cell depletion, are not known to induce type 1 IFN activity. Rather, type 1 IFN has been shown to induce BAFF production (37).

In summary, rituximab treatment for primary Sjögren's syndrome in this small, open-label trial was associated with no unexpected toxicities, except possibly exaggerated vaccine reactions. It only led to modest improvements in symptoms and no beneficial changes in lacrimal or salivary gland function. In addition to detailed studies of blood B cell subsets, exploratory analyses of gene transcripts and pathways in the peripheral blood suggest rituximab therapy substantially alters B cells, while having little impact on the IFN signature. Larger randomized, placebo-controlled clinical trials such as those reported by Devauchelle-Pensec and colleagues (34) are needed to further evaluate the clinical efficacy and safety of rituximab therapy for primary Sjögren's syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bowman SJ, Ibrahim GH, Holmes G, Hamburger J, Ainsworth JR. Estimating the prevalence among Caucasian women of primary Sjögren's syndrome in two general practices in Birmingham, UK. *Scand J Rheumatol*. 2004; 33:39–43. [PubMed: 15124941]
2. Fox RI. Sjögren's syndrome. *Lancet*. 2005; 366:321–331. [PubMed: 16039337]
3. Ramos-Casals M, Solans R, Rosas J, Camps MT, Gil A, del Pino-Montes J, et al. Primary Sjögren's syndrome in Spain. Clinical and immunologic expression in 1010 patients. *Medicine*. 2008; 87:210–219. [PubMed: 18626304]
4. Theander E, Henriksson G, Ljungberg O, Mandl T, Manthorpe R, Jacobsson LTH. Lymphoma and other malignancies in primary Sjögren's syndrome: a cohort study on cancer incidence and lymphoma predictors. *Ann Rheum Dis*. 2006; 65:796–803. [PubMed: 16284097]
5. Ramos-Casals M, Tzioufas AG, Stone JH, Sis3 A, Bosch X. Treatment of primary Sjögren's syndrome. A systematic review. *JAMA*. 2010; 304:452–460. [PubMed: 20664046]

6. Pijpe J, van Imhoff GW, Spijkervet FKL, Roodenburg JLN, Wolbink GJ, Mansour K, et al. Rituximab treatment in patients with primary Sjögren's syndrome. An open-label phase II study. *Arthritis Rheum.* 2005; 52:2740–2750. [PubMed: 16142737]
7. Devauchelle-Pensec V, Pennec Y, Morvan J, Pers J-O, Daridon C, Jouse-Joulin S, et al. Improvement of Sjögren's syndrome after two infusions of rituximab (anti-CD20). *Arthritis Rheum.* 2007; 57:310–317. [PubMed: 17330280]
8. Dass S, Bowman SJ, Vital EM, Ikeda K, Pease CT, Hamburger J, et al. Reduction of fatigue in Sjögren's syndrome with rituximab: results of a randomized, double-blind, placebo-controlled pilot study. *Ann Rheum Dis.* 2008; 67:1541–1544. [PubMed: 18276741]
9. Meijer JM, Meiners PM, Vissink A, Spijkervet FKL, Abdulahad W, Kamminga N, et al. Effectiveness of rituximab treatment in primary Sjögren's syndrome. A randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* 2010; 62:960–968. [PubMed: 20131246]
10. Abdulahad WH, Meijer JM, Kroese FGM, Meiners PM, Vissink A, Spijkervet FKL, et al. B cell reconstitution and T helper cell balance after rituximab treatment of active primary Sjögren's syndrome. *Arthritis Rheum.* 2011; 63:1116–1123. [PubMed: 21225693]
11. Edwards JCW, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med.* 2004; 350:2572–2581. [PubMed: 15201414]
12. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, Keystone EC, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy. *Arthritis Rheum.* 2006; 54:2793–2806. [PubMed: 16947627]
13. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med.* 2010; 363:221–232. [PubMed: 20647199]
14. Youinou P, Devauchelle-Pensec V, Pers J-O. Significance of B cells and B cell clonality in Sjögren's syndrome. *Arthritis Rheum.* 2010; 62:2605–2610. [PubMed: 20496425]
15. Hansen A, Gosemann M, Pruss A, Reiter K, Ruzickova S, Lipsky PE, et al. Abnormalities in peripheral B cell memory of patients with primary Sjögren's syndrome. *Arthritis Rheum.* 2004; 50:1897–1908. [PubMed: 15188366]
16. Hansen A, Gosemann M, Pruss A, Reiter K, Ruzickova S, Lipsky PE, et al. Abnormalities in peripheral B cell memory of patients with primary Sjögren's syndrome. *Arthritis Rheum.* 2004; 50:1897–1908. [PubMed: 15188366]
17. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis.* 2002; 61:554–8. [PubMed: 12006334]
18. Vivino FB, Al-Hashimi I, Khan Z, LeVeque FG, Salisbury PL, Tran-Johnson TK, et al. Pilocarpine tablets for the treatment of dry mouth and dry eye symptoms in patients with Sjögren's syndrome. A randomized, placebo-controlled, fixed-dose, multicenter trial. *Arch Intern Med.* 1999; 159:174–181. [PubMed: 9927101]
19. Talamo J, Frater A, Gallivan S, Young A. Use of the short form (SF36) for health status measurement in rheumatoid arthritis. *Br J Rheumatol.* 1997; 36:463–469. [PubMed: 9159541]
20. Abdallah KO, Luning Prak ET. B cell monitoring of transplant patients treated with anti-CD20. *Clin Transpl.* 2006:427–437. [PubMed: 18365400]
21. Sutter JA, Kwan-Morley J, Dunham J, Du YZ, Kamoun M, Albert D, et al. A longitudinal analysis of SLE patients treated with rituximab (anti-CD20): factors associated with B lymphocyte recovery. *Clin Immunol.* 2008; 126:282–290. [PubMed: 18226586]
22. Gao J, Cha S, Jonsson R, Opalko J, Peck AB. Detection of anti-type 3 muscarinic acetylcholine receptor antibodies in the sera of Sjögren's syndrome patients by use of a transfected cell line assay. *Arthritis Rheum.* 2004; 50:2615–2621. [PubMed: 15334476]
23. Van Gelder RN, von Zastrow ME, Yool A, Dement WC, Barchas JD, Eberwine JH. Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc Natl Acad Sci USA.* 1990; 87:1663–7. [PubMed: 1689846]

24. Sekiguichi DR, Smith SB, Sutter JA, Goodman NG, Propert K, Louzoun Y, et al. Circulating lymphocyte subsets in normal adults are variable and can be clustered into subgroups. *Cytometry B Clin Cytom.* 2011; 80B:291–299.
25. Waterman SA, Gordon TP, Rischmueller M. Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjögren's syndrome. *Arthritis Rheum.* 2000; 43:1647–1654. [PubMed: 10902771]
26. Park K, Haberberger RV, Gordon TP, Jackson MW. Antibodies interfering with the type 3 muscarinic receptor pathway inhibit gastrointestinal motility and cholinergic neurotransmission in Sjögren's syndrome. *Arthritis Rheum.* 2011; 63:1426–1434. [PubMed: 21312189]
27. Mackay F, Figgett WA, Saulep D, Lepage M, Hibbs ML. B-cell stage and context-dependent requirements for survival signals from BAFF and the B cell receptor. *Immunol Rev.* 2010; 237:205–225. [PubMed: 20727038]
28. Groom J, Kalled SL, Cutler AH, Olson C, Woodcock SA, Schneider P, et al. Association of BAFF/BlyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J Clin Invest.* 2002; 109:59–68. [PubMed: 11781351]
29. Pers J-O, Devauchelle V, Daridon C, Bendaoud B, Le Berre R, Bordron A, et al. BAFF-modulated repopulation of B lymphocytes in the blood and salivary gland of rituximab-treated patients with Sjögren's syndrome. *Arthritis Rheum.* 2007; 56:1464–1477. [PubMed: 17469105]
30. Cambridge G, Stohl W, Leandro MJ, Migone T-S, Hilbert DM, Edwards JCW. Circulating levels of B lymphocyte stimulator in patients with rheumatoid arthritis following rituximab treatment. Relationships with B cell depletion, circulating antibodies, and clinical relapse. *Arthritis Rheum.* 2006; 54:723–732. [PubMed: 16508933]
31. Emamian ES, Leon JM, Lessard CJ, Grandits M, Baechler EC, Gaffney PM, Segal B, Rhodus NL, Moser KL. Peripheral blood gene expression profiling in Sjogren's syndrome. *Genes Immun.* 2009; 4:285–296. [PubMed: 19404300]
32. Lavie F, Miceli-Richard C, Ittah M, Sellam J, Gottenberg J, Mariette X. Increase of B cell-activating factor of the TNF family (BAFF) after rituximab treatment: insights into a new regulating system of BAFF production. *Ann Rheum Dis.* 2007; 66:700–703. [PubMed: 17040963]
33. Bingham CO III, Looney RJ, Deodhar A, Halsey N, Greenwald M, Coddling C, et al. immunization responses in rheumatoid arthritis patients treated with rituximab. Results from a controlled clinical trial. *Arthritis Rheum.* 2010; 62:64–74. [PubMed: 20039397]
34. Devauchelle-Pensec, V., et al. Tolerance and efficacy of rituximab in primary Sjögren's syndrome: Final results of a randomized controlled trial. ACR/ARHP Annual Meeting; Washington, DC. November 10–14, 2012; p. abstract 2554
35. Seror R, Sordet C, Guillemin L, Hachulla E, Masson C, Ittah M, et al. Tolerance and efficacy of rituximab and changes in serum B cell biomarkers in patients with systemic complications of primary Sjögren's syndrome. *Ann Rheum Dis.* 2007; 66:351–357. [PubMed: 16950808]
36. Vosslander S, Raterman HG, van der Pouw Krann TCTM, Schreurs MWJ, von Blomberg BME, Nurmohamed MT, et al. Pharmacological induction of interferon type I activity following treatment with rituximab determines clinical response in rheumatoid arthritis. *Ann Rheum Dis.* 2011; 70:1153–1159. [PubMed: 21444302]
37. Brkic Z, Maria NI, van Helden-Meeuwse CG, van de Merwe JP, van Daele PL, Dalm VA, et al. Prevalence of interferon type 1 signature in CD14 monocytes of patients with Sjögren's syndrome and association with disease activity and BAFF gene expression. *Ann Rheum Dis.* (Published Online First 26 June 2012). 10.1136/annrheumdis-2012-201381

Figure 1. Total numbers of blood CD19⁺ B cells before and after treatment with rituximab
 Absolute B cell counts were obtained by multiplying the absolute lymphocyte count from the CBC by the CD19⁺ fraction by flow cytometry. Plotted are the CD19⁺ lymphocyte counts in cells per microliter of whole blood as a function of time in weeks. Time zero is the baseline assessment and corresponds to the day of the first rituximab infusion. Each patient is represented by a line with different symbols.

Figure 2. Analysis of blood B cell subsets following rituximab therapy in patients with primary Sjögren's syndrome

The average percentages of B cells in each of the different B-cell subsets (CD19⁺ lymphocytes) in all of the patients are plotted as a function of time in weeks following rituximab. Time zero is the baseline assessment and corresponds to the day of the first rituximab infusion. The subsets are defined on the basis CD27 and CD38 expression as described in the *Materials and Methods*.

Figure 3.

Figure 3A and B. Relationship between blood B cell depletion and serum BAFF levels.

A) median serum BAFF levels are significantly elevated above baseline at weeks 8 and 14 during the time of maximal blood B cell depletion and slowly decline to near baseline levels by week 52 in accordance with reconstitution of the blood B cell pool; B) the relationship between the numbers of blood B cells and serum BAFF levels is plotted for individual subjects at each of weeks 0, 8, 14, 26, 36, and 52.

Table 1**Patient characteristics**

Age (yr), median (range)	51 (34–69)
Females, %	100
Race, n (%)	
Caucasian	11 (91.7)
Black/African-American	1 (8.3)
Disease duration (yr), median (range)	8.0 (2–18)
Ocular symptoms, n (%)	12 (100)
Oral symptoms, n (%)	12 (100)
Ocular signs, n (%)	12 (100)
Schirmer-I test score, mm, median (range)	
OD	11 (0–35)
OS	5 (0–19)
Lissamine green staining test score (0–18), median (range)	
OD	9.0 (2–18)
OS	9.5 (4–18)
Unstimulated WSF rate, mL/min, median (range)	0.025 (0.0–0.22)
Stimulated WSF rate, mL/min, median (range)	0.05 (0.0–0.65)
Labial minor salivary gland focus score 1 per 4 mm ² , n	3/4
Fatigue (VAS > 50 mm), n (%)	10 (83.3)
Joint pain (VAS > 50 mm), n (%)	9 (75.0)
Severe parotid gland swelling, n (%)	3 (25.0)
Other extraglandular disease, n (%)	
Peripheral neuropathy	4 (33.3)
Interstitial lung disease	1 (8.3)
Anti-Ro (SSA) antibodies, n (%)	10 (83.3)
Anti-La (SSB) antibodies, n (%)	5 (41.7)
Rheumatoid factor, n (%)	10 (83.3)
SF-36, median (range)	
Physical function	60.0 (20, 100)
Mental function	70.0 (60, 90)

Abbreviations: WSF=whole salivary flow; VAS=visual analog scale; OD=right eye; OS=left eye

Table 2

Adverse events

	All Subjects (n=12)
Number of adverse events reported	162
Number of subjects with adverse events	12 (100 %)
Number of serious adverse events	2
Number of serious adverse events related to rituximab ^I	1
Number of subjects with serious adverse events	2 (16.7%)
Number of adverse events by severity	
Mild (grade 1)	138 (85.2%)
Moderate (grade 2)	20 (12.3%)
Severe (grade 3)	4 (2.5%)
Life-threatening (grade 4)	0
Fatal (grade 5)	0
Number of subjects with adverse events by severity	
Mild (grade 1)	5 (41.7%)
Moderate (grade 2)	5 (41.7%)
Severe (grade 3)	2 (16.7%)

^ISubject diagnosed with squamous carcinoma of the skin 301 days after rituximab therapy

Table 3

Analysis of peripheral blood B cell subsets before and after rituximab therapy

Subset	Time Point (week)	Median (cells/ μ l)	Range (min-max)
Transitional (CD38 ⁺⁺ , CD27 ⁻)	BL	21.24	1.5 – 47.7
	8	0.01	0.0 – 0.1
	14	0.01	0.0 – 0.2
	26	10.30	0.1 – 58.3
	36	13.89	0.5 – 46.8
	52	21.32	7.2 – 49.3
Mature naïve (CD38 ⁺ , CD27 ⁻)	BL	134.9	18.9 – 280.0
	8	0.04	0.0 – 0.2
	14	0.02	0.0 – 0.1
	26	0.57	0.1 – 74.8
	36	37.69	0.3 – 96.0
	52	75.94	1.4 – 192.7
Mature activated memory (CD38 ⁺ , CD27 ⁺)	BL	10.71	5.1 – 40.1
	8	0.18	0.0 – 0.5
	14	0.11	0.0 – 0.9
	26	0.60	0.3 – 2.0
	36	1.46	0.5 – 7.1
	52	2.16	1.2 – 23.6
Mature resting memory (CD38 ⁻ , CD27 ⁺)	BL	3.69	0.0 – 14.9
	8	0.02	0.0 – 0.1
	14	0.0	0.0 – 1.0
	26	0.10	0.0 – 0.5
	36	0.25	0.1 – 3.5
	52	0.36	0.1 – 8.4
Plasmablast (CD38 ⁺⁺ , CD27 ⁺⁺)	BL	1.01	0.3 – 3.9
	8	0.06	0.0 – 2.3
	14	0.06	0.0 – 0.5
	26	0.24	0.0 – 1.1
	36	0.43	0.0 – 5.0
	52	0.78	0.2 – 15.9
Double negative (CD38 ⁻ , CD27 ⁻)	BL	4.28	1.3 – 7.4
	8	1.54	0.0 – 3.6
	14	0.76	0.0 – 3.5
	26	0.87	0.0 – 6.5
	36	1.47	0.6 – 5.0
	52	1.28	0.4 – 8.7

Fresh whole blood (less than 24 hours old) was analyzed by flow cytometry as described in *Materials and Methods*. To calculate the B cell subset absolute cell counts the absolute lymphocyte count (in cells per microliter of whole blood) was obtained by Coulter counting or through the complete blood count at each of the local sites. The B cell fraction was obtained by multiplying the absolute lymphocyte count by the CD19+ fraction. B cell subset counts were computed by multiplying the B cell absolute count by the fraction of B cells in each of the subsets. The B cell subsets were defined using CD38 and CD27 staining into the following subsets: transitional (CD38⁺⁺, CD27⁻), mature naïve (CD38⁺, CD27⁻), mature activated memory (CD38⁺, CD27⁺), resting memory (CD38⁻, CD27⁺), plasmablast (CD38⁺⁺, CD27⁺⁺), and double negative (CD38⁻, CD27⁻), as described previously (21, 22). B cell subset data are not available for subject 08-096 at the baseline time point and not available for subject 04-098 at the week 26 time point. Time points are defined in weeks following rituximab administration.

BL = baseline.