Biochemical and Immunologic Effects of Rituximab in Patients With Primary Biliary Cirrhosis and an Incomplete Response to Ursodeoxycholic Acid

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The aim of this study was to determine the safety and potential efficacy of B-cell depletion with the anti-CD20 monoclonal antibody rituximab in patients with primary biliary cirrhosis (PBC) and an incomplete response to ursodeoxycholic acid (UDCA). This open-label study enrolled six patients with PBC and incomplete responses to UDCA to be treated with 2 doses of 1000 mg rituximab separated by 2 weeks and followed for 52 weeks. The primary endpoints were safety and changes in B-cell function. Two patients received only 1 dose of rituximab, one due to activation of latent varicella and the other due to a viral upper respiratory infection. Serum levels of total IgG, IgM, and IgA as well as anti-mitochondrial autoantibodies (AMAs) IgA and IgM decreased significantly from baseline by 16 weeks and returned to baseline levels by 36 weeks. Stimulation of B cells with CpG produced significantly less IgM at 52 weeks after treatment compared with B cells at baseline. In addition, transient decreases in memory B-cell and T-cell frequencies and an increase in CD25high CD4+ T cells were observed after treatment. These changes were associated with significant increases in mRNA levels of FoxP3 and transforming growth factor-β (TGF-β) and a decrease in tumor necrosis factor-α (TNF-α) in CD4+ T cells. Notably, serum alkaline phosphatase levels were significantly reduced up to 36 weeks following rituximab treatment.

Conclusion: These data suggest that depletion of B cells influences the induction, maintenance, and activation of both B and T cells and provides a potential mechanism for treatment of patients with PBC with an incomplete response to UDCA. (HEPATOLOGY 2012;55:512-521)

Primary biliary cirrhosis (PBC) is a female-predominant, organ-specific autoimmune disease characterized by nonsuppurative destructive cholangitis of the intrahepatic bile ducts.1,2 Both B-cell and T-cell responses to the E2 subunit of the inner mitochondrial membrane enzyme pyruvate dehydrogenase complex, E2 component (PDC-E2) have been documented in PBC and implicated in its pathogenesis.3-6 The serological hallmark of PBC is the presence of anti-mitochondrial autoantibodies (AMAs), especially to PDC-E2, which are present in 90%-95% of sera of patients with PBC. In addition, patients with PBC often have elevated serum levels of total immunoglobulin M (IgM) and B cells from patients with PBC produce significantly greater amounts of IgM when stimulated with CpG-B compared with healthy controls and patients with primary sclerosing cholangitis.8 Furthermore, treatment of transgenic mice expressing a dominant-negative transforming growth factor-β (TGF-β) receptor II (dnTGF-βRII), which develop autoimmune cholangitis and AMA, with anti-CD20 monoclonal antibody resulted in amelioration of liver inflammation, supporting a potential mechanism of action for B-cell–targeted therapies in PBC.9

Abbreviations: AIHA, autoimmune hemolytic anemia; AMA, anti-mitochondrial autoantibody; dnTGF-βRII, dominant-negative transforming growth factor-β receptor II; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; PBC, primary biliary cirrhosis; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PDC-E2, pyruvate dehydrogenase complex, E2 component; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; Treg, regulatory T cells; UDCA, ursodeoxycholic acid; WBC, white blood cell.

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Currently, therapy for PBC is limited to ursodeoxycholic acid (UDCA) and liver transplantation for end-stage disease. Although UDCA has demonstrated clinical benefits in liver biochemistries and potentially in survival, up to 40% of patients have a suboptimal response to UDCA and 10% will go on to die or require liver transplantation. Small clinical trials using immunosuppressants including corticosteroids, mycophenolate mofetil, azathioprine, and cyclosporine have either failed to show significant benefit or had unacceptable safety profiles. Based on our previous work in humans and mouse models implicating B cells in PBC pathogenesis, we hypothesized that targeted depletion of B cells would be an effective therapy with minimization of adverse effects.

Rituximab is a chimeric monoclonal antibody specific for human CD20 and a pan-B–cell surface marker, and depletes B cells by complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. Initially developed for the treatment of B-cell lymphoma, rituximab has since demonstrated efficacy for the treatment of several autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia (AIHA), pemphigus vulgaris, and others.

In this study, we report on an open-label study of six patients with PBC and incomplete responses to UDCA who were treated with rituximab. We evaluated safety, liver enzymes, and B-cell function at baseline and during the 52 weeks following treatment. Rituximab was well tolerated and led to decreases in serum immunoglobulins and AMA. In addition, the hyper-IgM response was no longer present in the repopulated B cells. Furthermore, there were decreases in the percentage of memory B and T cells and an increase in CD25\textsuperscript{high} regulatory CD4 T cells suggesting that depletion of B cells influences the induction, maintenance, and activation of T cells. Finally, we observed a significant reduction in serum alkaline phosphatase levels after rituximab treatment.

**Patients and Methods**

**Patients.** Female patients between the ages of 18 and 65 years diagnosed with PBC based on the presence of an AMA titer > 1:40, alkaline phosphatase at least twice the upper limit of normal, and liver histology compatible with stage I-III PBC, and who did not have normalization of their alkaline phosphatase after a minimum of 6 months of treatment with adequate doses of UDCA, were enrolled. Patients were excluded if they had evidence of decompensated liver disease (ascites, jaundice, coagulopathy, hepatic encephalopathy, or varices), other coexisting liver disease, treatment with immunosuppressive medications within 4 weeks of enrollment, or active infection. Permitted medications included prednisone of 10 mg daily or less and UDCA at a dose that was maintained at pre-enrollment doses.

**Study Design.** This was an open-label study conducted at a single academic clinical research center (ClinicalTrials.gov, Identifier: NCT00364819). After a screening visit, all subjects were treated with rituximab 1000 mg by intravenous infusion on days 1 and 15. Before rituximab infusion, patients received 100 mg of methylprednisolone intravenously. Safety assessments included a clinic visit and laboratory tests performed on the days of infusion as well as at 4, 8, 16, 24, 36, and 52 weeks as well as a liver biopsy at 52 weeks. Blood was also collected at each visit for B-cell and T-cell functional assays. In addition, the PBC-40 questionnaire, a validated tool for the assessment of quality of life in patients with PBC was administered before treatment and at week 52.

The study was initially planned to enroll 10 patients but was closed after six patients due to low enrollment. During the enrollment period, 24 patients with PBC and an incomplete response to UDCA were screened. Three subjects had cirrhosis and 15 subjects declined to participate. The study was approved by the Institutional Review Board, and all subjects gave written informed consent before enrollment.

**PBMC Isolation.** Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient using...
Histopaque-1077 (Sigma Chemical Co., St. Louis, MO), and the cells were washed and resuspended in phosphate-buffered saline (PBS) (Mediatech Inc., Herndon, VA) containing 0.5% bovine serum albumin (BSA; Fraction V, OmniPur; EMD Chemicals Inc., Gibbstown, NJ) and 0.05% EDTA (Sigma Chemical Co.). The viability of the cells was more than 98% by trypan blue dye exclusion.

**CD19**, **CD4**, and **CD8** Cell Isolation. Anti-CD19, anti-CD4, and anti-CD8 microbeads were used as recommended by the manufacturer (Miltenyi Biotech Inc., Auburn, CA). Briefly, CD19+ cells were first positively selected with an MS MiniMACS column (Miltenyi Biotech) from total PBMCs; the flow-through CD19− cell population was subjected to CD4+ T-cell positive separation. Furthermore, the flow-through CD19− CD4− cells were used to isolate CD8+ T cells. Each cell pellet was resuspended in 500 μL RNAlater (Applied Biosystems, Foster City, CA).

**B-Cell Culture.** To stimulate B cells, 2.0 × 10^5 CD19+ B cells and 8.0 × 10^5 CD19− non-B cells with 2 μM CpG-B (Invivogen, San Diego, CA) were cultured in 48-well flat-bottomed plates in 500 μL RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco-Invitrogen Corp., Grand Island, NY), 100 μg/mL streptomycin, and 100 U/mL penicillin (Invitrogen) for 96 hours at 37°C in a 5% CO₂ humidified atmosphere. After 96 hours of culture, supernatants were collected and clarified by centrifugation.

**Flow Cytometry.** PBMCs from patients with PBC were resuspended in staining buffer (0.2% BSA, 0.04% EDTA, 0.05% sodium azide in PBS), divided into 25-μL aliquots, and incubated with anti-human FcR blocking reagent (e Bioscience, San Diego, CA) for 15 minutes at 4°C. The cells were then washed and stained with the following antibodies for 30 minutes at 4°C:

- Fluorescein isothiocyanate-conjugated (FITC)-anti-CD4 (BD Pharmingen, San Diego, CA)
- CD8 (BD Pharmingen)
- FITC-anti-CD20 (eBioscience)
- Phycoerythrin-conjugated (PE)-anti-CD45RO (BD Pharmingen)
- PE-anti-CD38 (eBioscience)
- PE-Cy-Chrome (PE-Cy5)-anti-CD56 (BD Pharmingen)
- TRI-COLOR (TC)-anti-CD25 (Invitrogen/Caltag, Carlsbad, CA)
- Allophycocyanin-conjugated (APC)-antiCD19 (eBioscience)
- Alexa Fluor 750 (AF750)-conjugated-anti-CD27 (eBioscience). IgG isotype controls were used for negative controls. The cells were then washed once with PBS containing 0.2% BSA. After staining, the cells were washed and fixed with 1% paraformaldehyde in PBS. For analysis, stained cells were counted on a FACScan flow cytometer (BD Immunocytometry Systems) that had been upgraded by Cytek Development (Fremont, CA) to allow for five-color analysis. The acquired data were analyzed with Cellquest PRO software (BD Immunocytometry Systems).

**Real-Time PCR.** Total RNA was extracted using the MagMAX-96 Total RNA Isolation Kit (Applied Biosystems). One million cells of total RNA was reverse-transcribed with SuperScript III Reverse Transcriptase (Invitrogen) and oligo dT²⁰ primer (Invitrogen), and quantified on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Amplification was performed for 40 cycles in a total volume of 12 μL, and products were detected using RT² SYBR Green (SABiosciences, Frederick, MD). The relative expression level of each target gene was determined by normalizing its mRNA level to an internal control gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH; PPH00150E; SABiosciences). The primer sets for real-time polymerase chain reaction (PCR) were: Granzyme A (F) 5’ TATCCATGCTATGACCCAGCC 3’; Granzyme A (R) 5’ TTCACATCATCCCCCTTTTTTAGG 3’; Perforin (F): 5’ AGGAAGCTGGCCAGAAGCCAAAGA 3’; and Perforin (R): 5’ CACCATAGAGGCTCAAGGGAA GG 3’. These two primers were synthesized by Sigma Life Science (St. Louis, MO). FoxP3 (PPH00029B; SABiosciences), interleukin (IL)-4 (PPH00508A; SABiosciences), IL-10 (PPH00572B; SA Biosciences), IL-21 (PPH01684A; SABiosciences), IL-22 (PPH01079B; SABiosciences), TGF-β (PPH00508A; SABiosciences), and tumor necrosis factor-α (TNF-α; PPH00341E; SABiosciences) primer sets were purchased from SABiosciences.

**Serum Immunoglobulins and AMAs.** Levels of plasma IgG, IgM, and IgA were determined using the human IMMUNO-TEK IgG, IgM, and IgA enzyme-linked immunoabsorbent assay (ELISA) kit (ZeptoMetrix Corp., Buffalo, NY). Plasma levels and culture supernatant levels of anti–PDC-E2 were quantified using an ELISA. Briefly, 96-well ELISA plates were coated with purified recombinant PDC-E2 at 5 μg/mL in carbonate buffer (pH 9.6) at 4°C overnight, washed five times with Tris-buffered saline Tween-20 (TBS-T), and blocked with 5% skim milk in TBS for 30 minutes. Then 100 μL of the samples was added to individual wells of this microtiter plate for 1 hour at
room temperature (RT), and the plates were rewashed. Then 100 µL of horseradish peroxidase (HRP)-conjugated anti-human immunoglobulin (A+M+G) (H+L) (1:2000) or IgA (1:2000) or IgM (1:2000) or IgG (1:2000) (Zymed, San Francisco, CA) was added to each well for 1 hour at RT, and the microtiter wells were rewashed. Immunoreactivity was detected by measuring the optical density (O.D.) at 450 nm after exposure for 15 minutes to 100 µL of TMB peroxidase substrate (KPL, Gaithersburg, MD). Previously calibrated positive and negative standards were included with each assay.

Statistical Analysis. Values are expressed as the mean ± SEM. Statistical analysis was performed using a two-tailed Wilcoxon matched pairs test. Values with \( P < 0.05 \) were considered statistically significant.

Results

Safety of Rituximab in PBC. Six patients were enrolled and all received at least one infusion of rituximab (Table 1). Two patients received only 1 infusion of rituximab due to reactivation of Varicella zoster (patient 1) and an upper respiratory infection (patient 4), both of which resolved without complication. All patients completed 52 weeks of follow-up and otherwise tolerated the treatment well with no serious adverse events observed.

Immunoglobulin and AMA Levels. IgA, IgM, and IgG levels after rituximab treatment are shown in Fig. 1. After B-cell depletion by rituximab treatment, plasma levels of IgA, IgM, and IgG decreased. This decrease was sustained until week 24. At week 36 immunoglobulin levels began to recover. The largest decrease was seen in IgM levels: at week 24 IgM levels had decreased by almost 50% (0 weeks: 1.64 ± 0.20 mg/mL; 24 weeks: 0.88 ± 0.14 mg/mL).

Plasma reactivity against PDC-E2 (AMA) was positive in all patients before rituximab treatment. The pattern of change in the overall titers of AMA (Fig. 2A) after rituximab treatment was similar to that of the immunoglobulins (Fig. 2). Total AMA titers were significantly decreased at weeks 16 and 24 (Fig. 2A), and then at week 36 AMA titers began to increase. One patient, patient 5, became negative for AMA. Subtypes of AMA are shown in Fig. 2B-D.

Functional Analysis of B Cells. We next investigated the ability of the regenerating B cells to produce...
immunoglobulin when stimulated with 2 μM of CpG-B. Secreted immunoglobulin and AMA in the supernatant were assayed by ELISA after 4 days of culture with CpG-B (Fig. 3). IgM secretion by B cells isolated from patients after rituximab treatment was significantly lower than those before treatment (0 weeks: 241.7 ± 101.4 ng/dL; 52 weeks: 30.1 ± 9.0 ng/dL) (Fig. 3B). Although not significant, IgG and AMA secretion also decreased after rituximab treatment (Fig. 3C). The changes in AMA production during the culture period were associated with changes in the number of CpG-B-stimulated AMA-producing B cells, which had decreased (patient 3) or were not present (patient 2).

**Phenotypic Analysis of Lymphocytes.** To examine the effects of rituximab on lymphocytes, were analyzed lymphocyte subsets in PBMCs by flow cytometry (Table 2). Rituximab treatment was associated with nearly complete depletion of peripheral blood B cells (CD19+ cell) by week 2, and the B-cell count remained low through week 24 (0 weeks: 2.90 ± 0.76 × 10⁹ cells/mL; 2 weeks: 0.01 ± 0.00 × 10⁹ cells/mL; 16 weeks: 0.11 ± 0.09 × 10⁹ cells/mL; 24 weeks: 0.41 ± 0.16 × 10⁹ cells/mL). Total white blood cells (WBCs) and total lymphocytes were significantly decreased by rituximab treatment at week 24. The numbers of CD4+ and CD8+ T cells and CD56+ natural killer (NK) cells did not change significantly during the follow-up period.

As expected, rituximab treatment resulted in a decrease in the percentage of memory B cells in the CD19+ B-cell compartment and repopulation occurred with an increase in the percentage of immature bone marrow CD20+CD38+ B cells compared with CD19+CD27+ memory B cells²⁷ (Fig. 4A-a, 4B-a, 4A-b, 4B-b).

The percentage of regulatory T (Treg) cells identified as CD25high CD4+ was significantly increased at 16 weeks after rituximab treatment and steadily decreased (Fig. 4A-c and 4B-c).²⁸ Interestingly, the changes in Treg cells mirrored the changes in CD19+ B cells (compare Table 2 and Fig. 4B-c). Rituximab treatment also resulted in a transient decrease in the percentage of memory CD4+ and memory CD8+ cells in the T cells (Fig. 4A-d, 4B-d, 4A-e, 4B-e).

**T-Cell Messenger RNA Expression.** We analyzed forkhead box P3 (FoxP3) RNA expression in CD4+ T cells to confirm that the increase in the CD25high CD4+ population was truly a T reg cell subset. The expression of FoxP3 mRNA by CD4+ T cells at week 24 was also significantly higher compared with baseline (Fig. 5A) supporting the findings of the flow cytometry data and indicating that the CD25high CD4+ T cells are a regulatory subset of T cells.

Next, we analyzed the mRNA of several cytokines from CD4+ T cells (Fig. 5B-G). A significant increase in TGF-β expression (at 36 weeks) was observed. There was a trend toward an increase in IL-10 expression but this did not reach statistical significance. In addition, there was a transient decrease in TNF-α at week 24. This profile approximately mirrors the depletion and recovery of the B-cell compartment after rituximab treatment. Finally, we analyzed the expression of the cytotoxic T-cell granule proteins granzyme A and perforin but found no significant differences (Fig. 5H,I).

**Clinical Response.** Although this study was not designed to determine clinical efficacy, we analyzed the effects of rituximab on liver biochemistries (Fig. 6). The mean serum alkaline phosphatase was significantly decreased at 2, 24, and 36 weeks (294.7 ± 51.1, 206.7
In this study, we examined the safety and immunologic effects of selective B-cell depletion using the anti-CD20 monoclonal antibody rituximab, in patients with PBC and an incomplete response to UDCA. During a 52-week follow-up period, we assessed liver enzyme levels, antibody levels, and lymphocyte populations, with special emphasis on T-cell and B-cell subsets. Our results suggest that rituximab is safe, transiently reverses several of the immunologic abnormalities characterized in PBC, and may have potential therapeutic effect in this difficult to treat PBC population.

Although PBC is a relatively homogeneous disease in terms of demographics (middle-aged women) and autoantibodies (AMAs), disease severity and response to UDCA is markedly heterogeneous. Several studies have documented that subgroups of patients with PBC without a biochemical response to UDCA are at greater risk of disease progression, demonstrating that there is a need for new therapies.11,29,30

B-cell depletion has the potential to ameliorate autoimmune disease by decreasing autoantibody production as well as by decreasing antigen presentation by B cells. Several studies, on subjects such as antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV),31 IgM antibody–associated polynuropathies,32 RA,33 and SLE,34 have reported that B-cell depletion with rituximab resulted in reduced levels of autoantibodies and alleviation of these autoimmune diseases in patients. In the current study, we observed declines in autoantibodies, suggesting that autoreactive plasma cells can be eradicated if their B-cell progenitors are eliminated. In our study, the titer of AMAs decreased significantly, especially IgA and IgM AMA, but only transiently, suggesting that repeated treatment would be required and also suggesting the possibility that complete removal of the progenitor cells could

**Table 2. Changes in the Number of T Cells, B Cells, and Natural Killer (NK) Cells in Peripheral Blood**

<table>
<thead>
<tr>
<th>Mononuclear Cells (PBMCs)*</th>
<th>Baseline (Weeks After Initial Treatment)</th>
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<tbody>
<tr>
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<td>0w</td>
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<tr>
<td>Total WBCs, (\times 10^9)/mL</td>
<td>6.72 ± 0.84</td>
</tr>
<tr>
<td>Total lymphocytes, (\times 10^9)/mL</td>
<td>2.45 ± 0.43</td>
</tr>
<tr>
<td>CD4-positive cells, (\times 10^9)/mL</td>
<td>8.66 ± 1.68</td>
</tr>
<tr>
<td>CD8-positive cells, (\times 10^9)/mL</td>
<td>4.18 ± 1.11</td>
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<tr>
<td>CD19-positive cells, (\times 10^9)/mL</td>
<td>2.90 ± 0.76</td>
</tr>
<tr>
<td>CD56-positive cells, (\times 10^9)/mL</td>
<td>2.11 ± 0.48</td>
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The numbers of white blood cells (WBCs), lymphocytes, and CD20+ , CD4+ , CD8+ , and CD56+ cells in the PBMCs were measured at 0 weeks (baseline), 2 weeks, 16 weeks, 24 weeks, 36 weeks, and 52 weeks after the first treatment with rituximab; each percentage of subpopulation was analyzed by flow cytometer. Data are presented as the number of cells/mL ± SEM.†, P < 0.05 in two-tailed Wilcoxon matched pairs test.
result in eradication of the AMA-secreting plasma cells.

We note that although a pathogenic role for AMAs in PBC remains to be firmly established, a possible mechanism of AMA pathogenesis in PBC is that during transcytosis of AMA through biliary epithelial cells, exposure to the antibody may result in caspase activation and subsequent cell death and bile duct damage.\(^{35}\) In addition, we recently reported that coculture of serum AMAs with PBC macrophages and biliary epithelial cell apoptotic blebs results in a significant increase in proinflammatory cytokine secretion.\(^{36}\) These data suggest that depletion of AMA-secreting plasma cells could directly inhibit this hyperactive immune response and improve PBC by depressing the levels of cytotoxic and inflammatory agents at the site of bile duct injury.

In addition to the role of B cells and autoantibodies, T cells have also been implicated in PBC pathogenesis. Notably, the frequency of CD4\(^+\)CD45RO\(^+\) memory T cells has been found to be significantly higher in patients with PBC compared with normal controls,\(^{37,38}\) and T-cell clones with PDC-E2 specificity derived from patients with PBC were all CD4\(^+\)CD45RO\(^+\) T cell.\(^{39}\) In addition to the memory CD4\(^+\) T cells, memory CD8\(^+\) T cells are increased in the mouse PBC model.\(^{40}\) An important feature of memory T cells is that they require lower affinity
interactions or lower amounts of antigen for activation than naive T cells. Rituximab treatment has been shown to be able to diminish both CD4+ and CD8+ memory T cells in autoimmune diseases, and recovery of memory T cells appears to be associated with relapse of autoimmune disease. Our results also showed that rituximab treatment reduced the percentages of both CD4+ and CD8+CD45RO+ memory T cells (Fig. 5).

Not only do B cells differentiate into antibody-secreting plasma cells, they can also act as antigen-presenting cells capable of generating memory CD4+ T cells and autoreactive CD8+ T cells. B cells expressing membrane-bound anti–PDC-E2 could potentially function as antigen-presenting cells and generate autoreactive T cells to the PDC-E2 epitope. In our study we found that after treatment with rituximab there was a decrease in CD4+ and CD8+ memory cells, suggesting an additional mechanism of action involving the depletion of autoreactive B cells and subsequent reduction in activated autoreactive CD4+ and CD8+ T cells.

Several studies suggest that rituximab treatment of autoimmune diseases promotes expansion of the Treg cell compartment. However, the role of the expansion of Treg cells is not clear. Some data suggest that a decrease in autoreactive B cells leads to a decline in organ-specific and systemic inflammation, and this favors the emergence of regulatory T cells that prevent the reactivation of any remaining autoreactive cells. In keeping with these previous observations, we also observed an increase in the proportion of Treg cells, and this increase was coincident with increased expression of FoxP3 and TGF-β by the CD4+ T-cell compartment.

Finally, the results of this study demonstrated that rituximab is as safe and well tolerated in patients with PBC as in other indications and suggests that rituximab has a potential therapeutic effect in this subgroup of patients without an optimal response to UDCA. Although we have recently observed that B-cell depletion exacerbates liver disease in a xenobiotic mouse model of PBC, we saw no such evidence biochemically or histologically of disease acceleration in our study. Notably, in our mouse model, B-cell depletion was carried out before induction of disease with a xenobiotic, suggesting that B cells may have different roles in induction of PBC compared with propagation of PBC. Of primary importance was the decreased serum alkaline phosphatase, suggesting a decrease in bile duct injury. Interestingly, the three patients (patients 2, 3, and 6) who had the greatest decrease in alkaline phosphatase had a marked decrease in their memory B-cell
compartments. Moreover, two of these had dramatically decreased antibody production.

In summary, this study provides evidence for the safety and efficacy of rituximab for the treatment of patients with PBC and an incomplete response to UDCA. Our clinical outcome was a significant reduction of serum alkaline phosphatase after rituximab treatment. Multiple mechanisms were identified through which rituximab therapy may lead to clinical improvement of PBC, including reduction of serum AMA through depletion of memory B cells, increases in Treg cells, and modulation of cytokine production. Further clinical studies targeting B cells in this population are warranted.

References


