B Cell Depletion Enhances T Regulatory Cell Activity Essential in the Suppression of Arthritis

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B Cell Depletion Enhances T Regulatory Cell Activity Essential in the Suppression of Arthritis

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The efficacy of B cell-depletion therapy in rheumatoid arthritis has driven interest in understanding the mechanism. Because the decrease in autoantibodies in rheumatoid arthritis does not necessarily correlate with clinical outcome, other mechanisms may be operative. We previously reported that in proteoglycan-induced arthritis (PGIA), B cell-depletion inhibits autoreactive T cell responses. Recent studies in B cell-depletion therapy also indicate a role for B cells in suppressing regulatory mechanisms. In this study, we demonstrate that B cells inhibited both the expansion and function of T regulatory (Treg) cells in PGIA. Using an anti-CD20 mAb, we depleted B cells from mice with PGIA and assessed the Treg cell population. Compared to control Ab-treated mice, Treg cell percentages were elevated in B cell-depleted mice, with a higher proportion of CD4+ T cells expressing Foxp3 and CD25. On a per-cell basis, CD4+CD25+ cells from B cell-depleted mice expressed increased amounts of Foxp3 and were significantly more suppressive than those from control Ab-treated mice. The depletion of Treg cells with an anti-CD25 mAb concurrent with B cell-depletion therapy restored the severity of PGIA to levels equal to untreated mice. Although titers of autoantibodies did not recover

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heumatoid arthritis (RA) is a debilitating inflammatory disease of the synovial joints mediated by chronic activation of several different cell populations including T cells, B cells, neutrophils, and macrophages, although the precise contribution of each of these cell populations is unclear (1). There is a renewed interest in the involvement of B cells in RA based on the clinical efficacy of B cell-depletion therapy with anti-CD20 Ab (rituximab) (2, 3). B cell depletion reduces rheumatoid factor and anti-CCP Abs; however, variable decreases in these Abs irrespective of improvement in clinical disease activity suggests additional mechanisms for efficacy (4, 5). Removal of autoreactive B cells participating in Ag presentation, costimulation, and cytokine production likely play a role, but these have not been completely elucidated in RA (6). In other autoimmune diseases and in an animal model of autoimmune disease, the T cell compartment is altered after B cell depletion, resulting in reduced T cell activation and cytokine production (7–12).

Our murine model of RA, proteoglycan-induced arthritis (PGIA), is similar to human disease by several criterion including clinical assessment, radiographic analysis, scintigraphic bone scans, laboratory tests, and histological assessment of diarthrodial joints (13–18). In this model, proteoglycan (PG)-specific B cells are required as Ab-secreting cells as well as Ag-specific APCs (19). Secondary signals delivered by B cells through CD80/CD86 are essential for autoreactive T cell activation and the development of arthritis (20). B cells are required to maintain chronic inflammation as anti-CD20 mAb treatment inhibits arthritis, T cell proliferation, and cytokine production (12).

Autoreactive T cells that escape central tolerance are regulated in the periphery by T regulatory (Treg) cells (21). Treg cells are divided into natural Tregs that are induced in the thymus and inducible Treg cells that are activated in the periphery. Treg cells are characterized by high expression of CD25 and the transcription factor Foxp3, which is essential for Treg cell activity (22–24). Treg cells quell inflammation using several suppressive mechanisms that are mediated through both soluble and membrane-bound factors (25). In a number of autoimmune diseases, there are documented defects in Treg cell numbers and function that could potentially allow effector T (Teff) cell escape from suppression by the production of proinflammatory cytokines by either Teff cells or APCs (26–30). IFN-γ produced by Th1 cells and IL-6, IL-21, TGF-β produced by APCs that promote Th17 cells inhibit Treg cell differentiation (31–35). Thus, an inappropriate balance between Teff and Treg cells permits autoreactive responses.

A direct impact of B cells on Treg cell activity is not clearly defined, although some recent work suggests that there may be a link. In a mouse model of Crohn’s disease, B cells exacerbate ileitis through suppression of Treg cell function (36). Suppression of disease in B cell-depleted nonobese diabetes and thyroiditis is accompanied by an increase in CD25+Foxp3+ Treg cells (37–39). In clinical studies, Foxp3 mRNA transcripts and Treg cell numbers are elevated in lupus patients following B cell-depletion therapy, whereas in another study, Treg cells of patients with idiopathic thrombocytopenic purpura are defective prior to treatment, but had restored capacity to suppress after B cell-depletion
therapy (40, 41). Treg cells were also increased in rituximab-responsive cryoglobulinemia vasculitis patients (11). Based on these data, we were interested in determining if the Treg cells are involved in the suppression of arthritis after B cell depletion. In this study, we report that the inflammatory environment in autoimmune arthritis determines Treg cell activity. In B cell-depleted mice, there was a significant decrease in T eff eff activity that corresponded to an increase in the percentage of CD4+ CD25+ Foxp3+ T cells and in the level of Foxp3 protein expression. CD4+ CD25+ Treg cells from B cell-depleted mice were also significantly more effective at suppressing PG-specific CD4+ T cell proliferation than Treg cells from control mice. Treg cells contributed to the inhibition of PGIA as Treg cell suppression in B cell-depleted mice was completely abolished by the simultaneous depletion of Treg cells with anti-CD25 mAb. Recovery of arthritis severity in mice depleted of both B cells and Treg cells was accompanied by the restoration of PG-specific CD4+ T cell responses. These data demonstrate that the activity of B cells in autoimmune arthritis tips the balance toward inflammatory T cells and away from Treg cell control of inflammation.

Materials and Methods

**Mice, Ag, and assessment of arthritis**

BALB/c wild-type (WT) mice were obtained from the National Cancer Institute (Bethesda, MD). BALB/c IFN-γ−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). BALB/c B cell-deficient mice (B−/−) were provided by Dr. Mark Shlomchik (Yale University, New Haven, CT). Female mice (>3 mo) were immunized i.p. with 150 μg human PG in 2 mg dimethyliodoacetadyl (DDA)-ammonium bromide adjuvant (Sigma-Aldrich, St. Louis, MO) in 200 μl PBS (pH 7.2) and boosted on day 21 with 100 μg PG in DDA as previously described (42). Human cartilage PG was purified from joint replacement surgeries by procedures approved by the Institutional Review Board of Rush University Medical Center (Chicago, IL) as previously described (43). All animal experiments were approved by the Animal Care and Use Committee at Rush University Medical Center. Arthritic scores were assessed by blinded observers three times per week and evaluated by the extent of erythema and swelling of each paw on a scale of 0–4 providing a maximum score for each mouse of 16. Scoring of each paw was as follows: 0, normal; 1, mild erythema and swelling of several digits; 2, moderate erythema and swelling; 3, more diffuse erythema and swelling; and 4, severe erythema and swelling of complete paw with ankylosis. Arthritis score represents the mean ± SEM of the data.

**Abs, treatments, and B and Treg cell depletion**

B cell depletion was performed by a single i.v. injection of 250 μg anti-mouse CD20 (mCD20) mAb (18B12, IgG2a; Biogen Idec, San Diego, CA) or the control anti-human CD20 (hCD20) mAb (2B8; Biogen Idec), which has no cross-reactivity to the mouse CD20 molecule as described previously (12). Depletion of Treg cells was achieved by weekly i.p. injections of 500 μg anti-mouse CD25 mAb (PC 6.5.3, rat IgG1) (BioXCell, West Lebanon, NH) or control anti-HPRN mAb (rat IgG1) (BioXCell). All Ab treatments of PG-immunized mice began 4 d after the second PG-DDA immunization.

**Treg flow cytometry**

Spleen and lymph nodes of anti-mCD20 mAb and anti-hCD20 mAb–treated mice were harvested after PG-DDA immunization. Single-cell suspensions of each tissue were immunostained using anti-CD3 FITC, anti-CD4 PerCP-Cy5.5, and anti-CD25 allophycocyanin (BD Biosciences, San Jose, CA) with anti-Foxp3 PE (eBioscience, San Diego, CA). Using an FACSCanto II (BD Biosciences), stained cells were acquired and analyzed with FACSDiva software (BD Biosciences). Data represent the mean ± SEM.

**In vitro Treg cell suppression assays**

Spleens were harvested from untreated WT or WT treated with anti-mCD20 mAb or anti-hCD20 mAb mice after PG-DDA immunization. Splenic effector CD4+ T cells (Teff) from untreated arthritic WT mice were isolated using AutoMACS separation with negative selection microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Splenic Treg cells from arthritic mice treated with anti-mCD20 mAb or anti-hCD20 mAb were CD25+ positively selected from negatively isolated CD4+ T cells (Miltenyi Biotec). Varying concentrations of Treg cells (0–1.25 × 10^5) were cocultured with T cells (1.25 × 10^5) and mitomycin C-treated naive splenic APCs (2.5 × 10^5) with or without PG (10 μg/ml) in RPMI 1640 media containing 5% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (complete media) in quadruplicate in 96-well Falcon plates (Fisher Scientific, Fair Lawn, NJ). WT naive spleen cells were incubated at 1 × 10^5 cells/ml with 25 μg/ml mitomycin C (Sigma-Aldrich, St. Louis, MO) for 30 min to inactivate proliferation. Proliferation was measured by [3H]thymidine incorporation overnight of a 5-d culture. Data represent the mean ± SEM.

Detection of anti-PG Abs by ELISA

Mice were bled from the orbital plexus for serum at 4, 8, and 11 wk after the first PG-DDA immunization. Anti-mouse PG and anti-human PG Abs in serum samples were determined by ELISA. Individual mouse serum samples and internal standard samples (pooled sera from arthritic mice) were serially diluted (PBS/0.5% Tween-20) in enzyme immunoassay tissue-culture 96-half-area-well plates (Costar, Corning, NY) that were coated overnight with 0.5 μg human PG or 0.75 μg native mouse PG in carbonate buffer. Known concentrations of plate-bound unlabeled murine IgG1 and IgG2a Ab (Southern Biotechnology Associates, Birmingham, AL) without plate-bound PGs were used as standard curves. Unlabeled plate-bound Abs in standard curve wells or serum Abs bound to PG-coated wells were detected using HRP-labeled anti-mouse IgG1 or IgG2a (Zymed, San Francisco, CA) secondary Abs with o-phenylenediamine. Spectrophotometer readings at 490 nm determined colorimetric changes and concentrations of anti-PG serum Abs. Data represent the mean ± SEM.

**T cell proliferation and assessment of Th1 and Th17 cytokines**

Spleens were isolated from individual untreated mice or mice treated with: anti-mCD20 mAb; anti-hCD20 mAb; anti-mCD20 mAb with anti-CD25 mAb; anti-mCD20 mAb with rat IgG1 anti-HPRN mAb; anti-CD25 mAb; or rat IgG1 anti-HPRN mAb after PG-DDA immunizations. Isolated CD4+ T cells (2.5 × 10^5) as described above were cultured with mitomycin C–treated naive total spleen APCs (2.5 × 10^5) with or without PG (10 μg/ml) for 5 d. Proliferation was measured by [3H]thymidine incorporation as described above. Data represent the mean ± SEM. Supernatants were removed from similarly established cultures at day 4 and cytokines analyzed for IL-17 or IFN-γ concentrations, respectively, with ELISA kits (IFN-γ [BD Biosciences] or IL-17 [R&D Systems, Minneapolis, MN]) according to the manufacturer’s instructions. Data represent the mean ± SEM.

**Statistical analysis**

The Mann–Whitney U test was used to compare nonparametric data for statistical significance. Significance is based on a p value < 0.05.

**Results**

**Depletion of Treg cells prior to immunization but not after accelerates PGIA**

Previous studies have demonstrated that inflammatory conditions can limit Treg cell activity. To determine if the inflammatory environment in arthritis affects the function of Treg cells, we assessed Treg cell activity in PGIA. Treg cells were depleted prior to PG-DDA immunization or 4 d after the second PG-DDA immunization when anti-PG-specific B and T cell responses have developed (12). BALB/c mice were depleted of Treg cells by treatment with anti-CD25 mAb i.p. every 7 d. CD25+Foxp3+ Treg cells were depleted in the blood, spleen, and lymph nodes as measured by Foxp3+ and CD25+ staining of CD4+ cells (data not shown). Depletion of Treg cells 3 d prior to PG-DDA immunization resulted in the early onset of PGIA with enhanced arthritis severity (Fig. 1A, 1B). However, depletion of Treg cells 4 d after the second PG/DDA immunization at a time point when arthritis was initiated had no affect on PGIA (Fig. 1C, 1D). Although disease severity was not exacerbated, splenic CD4+ T cells from Treg cell-depleted mice displayed a significant increase in PG-specific proliferation and IFN-γ production than CD4+ T cells from control Ab-treated mice (Fig. 1E, 1F). However, the IL-17...
secretion from CD4+ T cells was suppressed (Fig. 1G) likely due to the ability of IFN-γ to suppress IL-17 production. These data indicate that Treg cells are able to inhibit already primed Teff cells. However, the inability of Treg cell depletion to exacerbate arthritis after immunization suggests that the number or potency of Treg cells may be reduced in the inflammatory environment. Alternatively arthritis is at maximum severity and cannot be further increased in absence of Treg cells.

CD4+CD25+ Treg cell number and function increase in B cell-depleted mice

B cell depletion is well documented to suppress autoimmune disease. Reduction in autoantibodies does not always correlate with inhibition of disease, suggesting other mechanisms may be involved. As described previously, depletion of B cells with anti-CD20 mAb treatment in early PGIA suppressed arthritis severity and inhibited PG-specific CD4+ T cell proliferation and IFN-γ and IL-17 cytokine production (12). Thus, the reduction in Teff cell responses may shift the balance away from Teff cells and toward Treg cells. To determine if the reduction in Teff cell activity in B cell-depleted mice was in part due to an increase in Treg cells, we analyzed spleens and lymph nodes of mice after treatment with either anti-CD20 mAb or control Ab. Titrated numbers of CD4+CD25+ Treg cells were cocultured with CD4+CD25+Teff cells from arthritic mice in the presence of PG and mitomycin C-treated naive total spleen cells with or without PG (10 μg/ml) for 4 d. Proliferation (E) of CD4+ T cells was measured by [3H]thymidine incorporation. Supernatants were harvested and assayed by ELISA for IFN-γ (F) and IL-17 (G). Values are mean ± SEM and representative of two independent experiments. *p < 0.05.

Because an increase in Foxp3 protein expression in Treg cells correlates with their suppressor capacity (44), we examined the suppression potency of Treg cells from B cell-depleted mice in a suppressor assays. CD4+CD25+ Treg cells were isolated from spleens of PG-immunized mice treated with either anti-CD20 mAb or control Ab. Titrated numbers of CD4+CD25+ Treg cells were cocultured with CD4+CD25− Teff cells from arthritic mice in the presence of PG and mitomycin C-treated naive splenocytes as APCs. Parallel to the increased levels of Foxp3 expression, CD4+CD25+ Treg cells from B cell-depleted arthritic mice suppressed PG-specific CD4+ T cell proliferation more efficiently at lower concentrations of Treg cells than those from control Ab-treated mice (Fig. 2F). At higher concentrations of Treg cells, a saturation effect on Teff cells by CD4+CD25+ cells was observed, as proliferation of Teff cells was similar for cultures containing CD4+CD25− Treg cells in B cell-depleted mice (Fig. 2G). These data demonstrate that B cell depletion results in an increase in the percentage and potency of Treg cells.

We next asked whether the change in Treg cells in B cell-depleted mice was a consequence of immune activation. In naive mice genetically deficient in B cells, there was the expected increase in the percentage of CD4+ T cells numbers, not an increase in Treg cell numbers (Fig. 2F), resulting in a reduction in the ratio of CD4+ T cells to CD4+CD25+Foxp3+ Treg cells in B cell-deficient mice (Fig. 2G).

FIGURE 1. Depletion of Treg cells prior to immunization but not after accelerates PGIA. BALB/c mice were immunized with PG in DDA twice with a 3-wk interval. Mice depleted of Treg cells with anti-CD25 mAb (n = 7) or treated with rat IgG1 (n = 7) were injected i.p. every 7 d beginning either 3 d prior to the initial PG-DDA immunization (A, B) or 4 d after the second PG-DDA immunization (C, D). Arthritis score (A, C) is the sum of paw inflammation scores divided by the number of arthritis mice. Incidence (B, D) is the percentage of mice with PGIA. At week 13 after the initial PG-DDA immunization, spleens were harvested. CD4+ T cells were purified from mouse spleens and cocultured with mitomycin C-treated naive total spleen cells with or without PG (10 μg/ml) for 4 d. Proliferation (E) of CD4+ T cells was measured by [3H]thymidine incorporation. Supernatants were harvested and assayed by ELISA for IFN-γ (F) and IL-17 (G). Values are mean ± SEM and representative of two independent experiments. *p < 0.05.
depleted mice is a consequence of inflammation. To further confirm in another system that reduction in inflammation results in an increase in Treg cells, we assessed Treg cells in PG-immunized IFN-\(\gamma^{2/2}\) mice. We have previously reported that PGIA is suppressed in IFN-\(\gamma^{2/2}\) mice (18, 43). In comparing Treg cells in WT and IFN-\(\gamma^{2/2}\) mice, we found the number and percentage of CD4\(^+\) CD25\(^+\)Foxp3\(^+\) cells were increased in IFN-\(\gamma^{2/2}\) mice (Fig. 3C, 3D). These data further support that inflammation suppresses Treg cells.

Simultaneous Treg cell and B cell depletion restores PGIA and the autoreactive CD4\(^+\) T cell reactivity

The increased suppressor phenotype of Treg cells suggests that the effectiveness of B cell depletion in PGIA may be in part due to enhanced Treg cell activity. To test this in vivo, we depleted Treg cells using anti-CD20 mAb treatment simultaneously with B cell depletion. The efficacy achieved with B cell depletion was completely reversed with the concomitant depletion of Tregs as mice treated with both anti-CD20 and anti-CD25 mAbs experienced a similar disease course as untreated WT mice (Fig. 4A, 4B). The absence of a resurgent autoantibody response in B cell-depleted mice with Treg cell depletion implicated the T cell compartment in the recovery of disease (data not shown). CD4\(^+\) T cells isolated from mice treated with anti-CD20 mAb and anti-CD25 mAb recovered their proliferative response to PG (Fig. 4C) as well as expression of IFN-\(\gamma\) and IL-17 (Fig. 4D, 4F) compared with mice treated with anti-CD20 mAb and rat IgG1 control Ab. These data demonstrate that Treg cells are a major contributor to the suppression of arthritis in B cell-depleted mice by reducing Teff cell responses.

Discussion

We and others have suggested that B cell-depletion therapy in autoimmune diseases is related to the suppression of autoreactive
Treg cells are the main mechanism by which Teff cells are controlled (46). Accordingly, reduced total numbers or decreased suppressor capacity of Tregs has been reported in several autoimmune diseases (46, 47). We found that in PGIA, depletion of Treg cells led to exacerbated arthritis when depleted before PG immunization but not after immunization. There are several possible explanations for these results: Treg cell potency or number may be reduced despite a clear increase in the Teff responses (Fig. 1). It is also possible that any exacerbation in arthritis due to Treg depletion could not be detected as arthritis was at maximum severity. This study was designed to determine if the mechanism for suppression of arthritis in B cell-depleted mice involves Treg cells.

The ability of Th subsets with established effector phenotypes to convert to anti-inflammatory subsets or Treg cells to convert to proinflammatory subsets is an area of intense research and debate. A recent article by Rubtsov et al. (48) demonstrates convincingly that expression of Foxp3 in committed Treg cells is stable under physiological and many inflammatory conditions. However, the authors do mention several situations in which Treg cells may lose Foxp3 expression, such as cells under certain stress conditions or those that newly or transiently expressed Foxp3. In fact, epigenetic studies demonstrated that many Th subsets differentiated in vitro maintain activating histone modifications of genes for master regulatory transcription factors responsible for other Th subset phenotypes (49). Adoptive transfer of Foxp3 expressing CD4+ T cells into T cell-deficient hosts demonstrated that Tregs are capable of losing Foxp3 expression and repopulating the T follicular helper cell compartment of Peyer’s patches in vivo (50). The conversion of Treg cells to this proinflammatory phenotype in this model of homeostatic repopulation required the presence of B cells and their expression of CD40 (50). We reasoned that the depletion of B cells and the reduction in Teff cell responses might shift the balance toward the differentiation of Treg cells. Examination of the Treg cells in B cell-depleted mice showed that the percentage and function of Treg cells increased (Fig. 2). Similar increases in Treg cell percentages and functions were noted in PGIA (Fig. 4). This suggests that the mechanism for suppression of arthritis in PGIA is not dependent on B cell depletion but rather on the presence of Treg cells.

FIGURE 3. Inflammation suppresses the number and percentage Treg cells. A and B, Spleen cells from naive WT or B cell-deficient mice (n = 4) were assessed for number and percentage of CD4+ T cells and CD4+CD25+Foxp3+ by flow cytometry. C and D, Spleen cells from PG-immunized WT and IFN-γ−/− mice (n = 4) were analyzed for number and percentage of CD4+ T cells and CD4+CD25+Foxp3+ by flow cytometry. Values are mean ± SD and representative of two independent experiments.*p ≤ 0.05.

FIGURE 4. Simultaneous Treg and B cell deletion restores PGIA and autoreactive CD4+ T cell reactivity. B cell-depleted mice were treated weekly with anti-CD25 mAb (n = 7) or rat IgG1 (n = 7) beginning on the same day as B cell depletion. Arthritis score (A) and incidence (B) of arthritis. Spleens were harvested 13 wk after the initial PG-DDA immunization. CD4+ T cells were purified from indicated mouse spleens and cocultured with mitomycin C-treated naïve total spleen cells with or without PG (10 μg/ml). Proliferation (C) of CD4+ T cells was measured by [3H]thymidine incorporation during the final 24 h of 5 d cultures. IFN-γ (D) and IL-17 (E) was measured by ELISA of supernatants of 4 d cultures. Values are mean ± SEM and representative of two independent experiments.*p ≤ 0.05.
cells were reported in B cell-depleted mouse models of NOD and thyroiditis (39). Therefore, it appears that B cells have a profound impact on Treg cell presence and effectiveness during inflammatory conditions. However, this observation is not universal. In experimental autoimmune encephalomyelitis, despite the reduction in Teff responses in B cell-depleted mice after MOG immunization, Tregs are not increased, and, in some cases, Treg cells are reduced (51, 52). There are numerous factors that are involved in the interplay among B cells, Teff cells, and Treg cells in different models (i.e., induced versus spontaneous disease models, strain of mouse, route of Ag exposure, timing of the B depletion, and timing of Treg analysis).

Although naïve B cell-deficient mice do not have increased numbers of Treg cells (Fig. 3) (29), the loss of B cells at the time of inflammation may result in reduced polarization of naive CD4+ T cells toward effector phenotypes and more toward regulatory populations. B cell depletion during PGIA results in significantly reduced PG-specific Th1 and Th17 activation (12). This reduction in the inflammatory cytokines IFN-γ and IL-17 during B cell depletion likely contributes to the increase in percentage of Treg cells in the spleens and lymph nodes of arthritic mice depleted of B cells (Fig. 2B, 2C). Studies show that Th1 IL-12, IFN-γ, and Th2 IL-4 can inhibit TGF-β-induced Treg cell differentiation of naive Th cells (53). Similarly, a deficiency in IFN-γ in PGIA results in reduction in arthritis and an increase in Treg cells. Another example in vivo is the inflammation elicited by Toxoplasma gondii infection, which induces a substantial Th1 response that limits Treg cell conversion from naive Th cells and the maintenance of their regulatory phenotype in vivo (54). The reduction in Th17 cells in B-depleted mice with PGIA may also affect Treg cells because in experimental autoimmune encephalomyelitis, the absence of IL-6 resulted in an overwhelming adaptive Treg cell response that suppressed the Ag-specific Th17 response and disease manifestation (55).

Along with the increase in the percentage of Treg cells, Foxp3 levels were elevated in CD4+CD25+ T cells from mice depleted of B cells (Fig. 2). The suppressive capabilities of Treg cells have been directly related to the level of Foxp3 transcripts expressed by these cells (44). Thus, the increase in Foxp3 expression in B cell-depleted mice indicated a concurrent amplification of suppressor function. In vitro proliferation assays confirmed this supposition, as CD4+CD25+ T cells from B cell-depleted mice were significantly more effective at suppressing proliferation of CD4+ T cells from arthritic mice in response to restimulation with PG (Fig. 2H).

In the clinic, similar findings have been observed with B cell-depletion therapy. Elevated levels of Foxp3 mRNA are detected in PBMCs from lupus patients treated with anti-CD20 mAb (14). Also, defective Treg cell suppression is restored in idiopathic thrombocytopenic purpura patients after B cell-depletion therapy (15). The findings that B cell depletion augments Treg cell Foxp3 expression and suppressor function support a Treg cell-dependent mechanism of B cell depletion efficacy.

In vivo studies using dual depletion of B cells and Treg cells resulted in a significant elevation in CD4+ T cell responsiveness to PG as measured by proliferation along with IFN-γ and IL-17 production (Fig. 4C-E). The return of PGIA in mice depleted of both B cells and Treg cells cannot be attributed to be a general response to Treg cell depletion, as mice treated with anti-CD25 alone did not experience exacerbated arthritis (Fig. 1C, 1D). Treg cell influence on PGIA has previously been described to be insufficient under normal circumstances and is believed to be the result of an uncontrolled PG-specific CD4+ T cell response (56). In support of this, Treg cell depletion prior to PG-DAI immunization and the activation of PG-specific CD4+ T cells in PGIA was exacerbated with an earlier onset (Fig. 1A, 1B). The reduced autoreactivity of the CD4+ T cells along with the strengthening of Treg cell populations in B cell-depleted mice allow Treg cells to control pathogenic inflammation of PGIA. Our demonstration that Treg cells function to support the efficacy of B cell depletion in autoimmune arthritis suggests that RA patients receiving B cell-depletion therapies may benefit from a concomitant therapy that promotes further Treg expansion such as IL-2/anti–IL-2 complexes (57, 58).

In this study, we demonstrate a role for B cells in limiting Treg cell suppressor activity in the promotion of inflammation in a mouse model of autoimmune arthritis, PGIA. In this model, B cells contribute to an inflammatory environment that inhibits Treg cell expansion and function and/or promotes the differentiation of naive CD4+ T cells preferentially to proinflammatory effector phenotypes rather than Treg cells. Alternatively, the efficiency of B cells to act as APCs may expand the pool of autoreactive Teff cells beyond the control of Treg cells. Further elucidation of the mechanisms used by B cells to suppress Treg cells will have a major impact on the development of new therapies for the treatment of chronic inflammatory diseases.

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Disclosures

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