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*J Immunol* published online 2 February 2015
http://www.jimmunol.org/content/early/2015/01/31/jimmunol.1402029

Supplementary Material
http://www.jimmunol.org/content/suppl/2015/01/31/jimmunol.1402029.DCSupplemental

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IFN-β Treatment Requires B Cells for Efficacy in Neuroautoimmunity

Ryan D. Schubert,† Yang Hu,‡,1 Gaurav Kumar,‡,1 Spencer Szeto,‡ Peter Abraham,* Johannes Winderl,* Joel M. Guthridge,‡ Gabriel Pardo,‡ Jeffrey Dunn,* Lawrence Steinman,* and Robert C. Axtell‡

IFN-β remains the most widely prescribed treatment for relapsing remitting multiple sclerosis. Despite widespread use of IFN-β, the therapeutic mechanism is still partially understood. Particularly, the clinical relevance of increased B cell activity during IFN-β treatment is unclear. In this article, we show that IFN-β pushes some B cells into a transitional, regulatory population that is a critical mechanism for therapy. IFN-β treatment increases the absolute number of regulatory CD19+CD24++CD38++ transitional B cells in peripheral blood relative to treatment-naive and Copaxone-treated patients. In addition, we found that transitional B cells from both healthy controls and IFN-β-treated MS patients are potent producers of IL-10, and that the capability of IFN-β to induce IL-10 is amplified when B cells are stimulated. Similar changes are seen in mice with experimental autoimmune encephalomyelitis. IFN-β treatment increases transitional and regulatory B cell populations, as well as IL-10 secretion in the spleen. Furthermore, we found that IFN-β increases autoantibody production, implicating humoral immune activation in B cell regulatory responses. Finally, we demonstrate that IFN-β therapy requires immune-regulatory B cells by showing that B cell-deficient mice do not benefit clinically or histopathologically from IFN-β treatment. These results have significant implications for the diagnosis and treatment of relapsing remitting multiple sclerosis. The Journal of Immunology, 2015, 194: 000–000.

Type I IFNs, which include IFN-β, elevate expression of BAFF, increase B cell activity, and drive the production of autoantibody in systemic lupus erythematosus (SLE) and neuromyelitis optica (NMO), promoting inflammation (1–3). In one sense, these are “type 1 IFN diseases” where B cell autoantibody production is clearly pathogenic. In relapsing remitting multiple sclerosis (RRMS), IFN-β also increases serum levels of BAFF and B cell activity (4, 5), yet in a seeming paradox, IFN-β reduces inflammation and decreases relapses (6). For 20 years, IFN-β has been the leading therapy for RRMS. Other studies have shown that IFN-β alters the function of T cells and myeloid cells in RRMS and experimental autoimmune encephalomyelitis (EAE) to reduce disease severity (7, 8). The experiments described in this article report a novel, previously unappreciated therapeutic mechanism for IFN-β in which therapy maintains a population of BAFF-dependent regulatory B cells that suppresses cell-mediated CNS inflammation.

Materials and Methods

Patient recruitment, PBMC isolation, and flow cytometry

RRMS patients and healthy volunteers were recruited and provided consent at Stanford Blood Center and Stanford Multiple Sclerosis Center or the Oklahoma Multiple Sclerosis Center of Excellence under Institutional Review Board–approved protocols. Patient disease diagnosis and activity were assessed by credentialed neurologists. PBMCs from healthy donors and RRMS subjects were isolated by centrifugation through Ficoll-Paque Plus (GE Life Sciences). PBMCs were frozen in 5% BSA and 10% DMSO before being thawed in a 37°C water bath. Cells were then washed with 1% FCS in PBS and stained with 10% human serum to block FcRs before incubation with the following anti-human Abs: FITC anti-CD40 (eBioscience), 40 nM CpG ODN (Invivogen), and brefeldin A (GolgiPlug, BD Biosciences) in combination with 1Y. H. and G.K. contributed equally to this manuscript.

Published February 2, 2015, doi:10.4049/jimmunol.1402029

Received for publication August 8, 2014. Accepted for publication December 23, 2014.

This work was supported by National Institutes of Health Grant R00 NS075099-04 (to R.C.A.). The multiple sclerosis biorepository at Oklahoma Medical Research Foundation, 825 NE 13th Street, A225.1, Oklahoma City, OK 73104. Y.H. and G.K. contributed equally to this manuscript.

Addendum

Abbreviations used in this article: EAE, experimental autoimmune encephalomyelitis; GA, glatiramer acetate; NMO, neuromyelitis optica; RRMS, relapsing remitting multiple sclerosis; SLE, systemic lupus erythematosus. Copyright © 2015 by The American Association of Immunologists, Inc. 0002-1767/15/525/00 1Y. H. and G.K. contributed equally to this manuscript.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1402029

The online version of this article contains supplemental material. For in vitro stimulation of PBMCs

In vitro stimulation of PBMCs

For intracellular FACS of IL-10 in B cell populations, we obtained fresh PBMCs from 5 IFN-β-treated MS patients and 5 healthy volunteers and cultured at 2.5 × 10^6 cells/ml with 3 μg anti-human Ig (Jackson Immunoresearch), 1 μg anti-human CD40 (eBioscience), 40 nM CpG ODN 2006 (Invivogen), and brefeldin A (GolgiPlug, BD Biosciences) in comb-
plete RPMI 1640 for 5 h, then surface stained with anti-CD19 PerCP-Cy5.5, anti-CD24 FITC, and anti-CD38 PE. Cells were then fixed, permeabilized using the intracellular FACS kit (BD Biosciences), and stained with anti-human IL-10 allophycocyanin (Biolegend). To assess secreted IL-10 by ELISA, we stimulated fresh PBMCs (2.5\times10^6 cells/ml) from three healthy volunteers with or without anti-human Ig, anti-human CD40, and CpG in the presence or absence of 1000 U/ml recombinant human IFN-β (PBL Interferon Source) for 72 h. IL-10 in culture supernatants were assessed by a human IL-10 ELISA Kit (eBioscience).

**EAE induction**

Eight- to ten-wk-old female C57BL6/J and muMT mice were induced with EAE by an immunization with 150 μg MOG p35–55 (Stanford) emulsified in CFA (4 mg/ml heat-killed M. tuberculosis) followed by an i.p. injection of 500 ng *Bordetella pertussis* toxin (Difco Laboratories) in PBS at the time of and 2 d after immunization. Paralysis was monitored daily using a standard clinical score range: 1) loss of tail tone, 2) incomplete hind-limb paralysis, 3) complete hind-limb paralysis, 4) forelimb paralysis, and 5) moribund/dead. Mice were treated every second day with 10,000 U/dose recombinant mouse IFN-β (PBL) or vehicle (0.5% albumin in PBS) from EAE days 6 to 20.

**In vitro stimulations of mouse spleen cells**

Single-cell suspensions of spleen cells from mice with EAE were cultured at 2.5 \times 10^6 cells/ml in complete RPMI 1640 with 1 μg/ml MOG35–55 peptide for 72 h. IL-10 from the culture supernatants were assessed by an IL-10 ELISA Kit (eBioscience).

**Passive transfer of B cells**

EAE mice treated with IFN-β or vehicle were sacrificed at EAE day 10, and B cells were isolated from spleen cells by magnetic sorting using anti-B220–conjugated magnetic beads (Miltenyi). A total of 20 \times 10^6 B cells from either IFN-β– or vehicle-treated donor mice were injected (i.p.) into recipient C57BL/6 mice at EAE day 6, and disease was monitored for 25 d.

**Histology**

Brains and spinal cords were dissected from EAE mice and the tissue was fixed in 10% formalin in PBS and embedded in a single paraffin block. Sections 8 μm thick were stained with H&E and Luxol fast blue, and imaged using a Nikon Eclipse E200 light microscope and a Nikon DS-V2 camera.

**Flow-cytometry analysis of EAE tissue**

Infiltrating cells were isolated from spinal cords from three to four perfused animals. CNS homogenates were incubated with collagenase and DNAse for 1 h at 37°C, and cells were purified by a Percoll gradient. Single-cell suspensions of spleen, draining lymph nodes, bone marrow, and infiltrating CNS cells were incubated with unconjugated anti-CD16/CD32, then stained with aqua live/dead stain and combinations of the following Abs: CD21-FITC, CD23-Biotin (Streptavidin-Qdot605), CD138-PE, IgM-PECy7, CD19-PECy5.5, CD43-Alexa 647, CD1d-PE. Cells were then analyzed on an LSRII.

**Serum BAFF and anti-MOG Ab detection**

Mouse BAFF was measured in plasma with the mouse BAFF ELISA kit (R&D). Levels of anti-MOG Abs in mouse plasma were performed using indirect ELISAs. In brief, ELISA plates were coated with 10 μg/ml MOG p35–55 peptide. Plates were probed with 1/100 dilutions of serum from individual mice, and reactive Abs were detected using peroxidase-conjugated goat anti-mouse specific for IgG or IgM (Southern Biotech) and developed with tetramethylbenzidine.

**Results**

**IFN-β therapy increases specific B cell populations in RRMS patients**

Because IFN-β treatment increases the amount of serum BAFF protein, a known survival factor for transitional B cells and plasma

**FIGURE 1.** IFN-β therapy increases transitional B cells in blood from RRMS patients. PBMCs from treatment-naive, IFN-β–treated, and GA-treated patients, as well as healthy controls, were analyzed using flow cytometry for the following: the frequency (A and B) and absolute number (C) of total CD19+ B cells; the frequency (D and E) and absolute number (F) of CD19+CD24++CD38++ transitional B cells; the frequency (G) of transitional 1 and 2 (T1 and T2) B cells; the frequency (H and I) and absolute number (J) of CD19+CD38+IgM−IgD− class-switched memory B cells; and the frequency (K and L) and absolute number (M) of CD19+CD27+CD38++ plasmablasts. Statistical significance was determined by one-way ANOVA (**p < 0.01, *p < 0.05).
blasts/cells in humans (9), we hypothesized that these B cell subsets would be increased in the peripheral blood of RRMS patients taking IFN-β. Under an Institutional Review Board–approved protocol, we recruited patients who were either treatment naive or actively using a prescribed preparation of recombinant IFN-β or Copaxone (GA) for peripheral blood draw. The RRMS patients gave their written informed consent for this study, and were similar in age and sex (Supplemental Table I).

In our patient cohort, there was a trend that serum BAFF protein, measured by ELISA, was elevated in patients taking IFN-β compared with patients taking GA, but the difference was not statistically significant (IFN-β: 819.3 ± 92.8 pg/ml; GA: 691.9 ± 23.8 pg/ml; data not shown). However, we did observe that IFN-β–treated RRMS patients had a statistically significant increase in the frequency of CD19⁺ B cells relative to treatment naive and GA-treated MS patients and to healthy controls (Fig. 1A, 1B). There was also a significant increase in the absolute numbers of B cells in the IFN-β–treated MS patients compared with the treatment-naive patients (Fig. 1C).

We then assessed the transitional B cell population defined by surface CD19⁺CD24⁺⁺CD38⁺⁺ (Fig. 1D), a population of B cells with immune-regulatory function in humans (10). We found that both the frequency and the absolute numbers of transitional B cells were increased in the IFN-β–treated patients compared with the treatment-naive and GA MS patients (Fig. 1E, 1F). We further subdivided transitional B cells into the transitional 1 and 2 subsets, T1 and T2, by expression of IgM, IgD (Fig. 1G). We found the absolute numbers of T1 and T2 B cells were significantly increased in IFN-β–treated patients compared with treatment-naive MS patients (Supplemental Fig. 1A, 1B).

We also assessed other B cell subsets in our cohort of RRMS patients. The frequency, but not absolute number, of class-switched memory B cells, represented by CD19⁺CD38⁺⁺⁺IgM⁺IgD⁺ (Fig. 1H), was significantly decreased in IFN-β–treated patients compared with treatment-naive and GA patients (Fig. 1I, 1J). Finally, we observed a statistically significant increase in the absolute number but not frequency of plasmablasts, defined as CD19⁺CD27⁺⁺CD38⁺⁺ (Fig. 1K) (11), in patients taking IFN-β compared with treatment-naive MS patients (Fig. 1K–M). In summary, the data show that transitional B cells and plasmablasts are increased in the peripheral blood during IFN-β treatment compared with treatment-naive patients, whereas class-switched memory cells are not. GA-treated patients did not demonstrate these changes. Therefore, the observed changes in B cells in IFN-β–treated patients were likely due to IFN-β treatment and not a general feature of MS disease in remission.

**Transitional B cells are a major source of IL-10 in MS patients treated with IFN-β**

It has recently been shown that the transitional B cell population has a regulatory function in healthy individuals and is capable of producing large amounts of IL-10 that may suppress Ag-mediated T cell activity in healthy individuals (10). Yet in lupus, transitional B cells lack IL-10–dependent regulatory function and instead drive both effector T cell function and antinuclear autoantibody production (10). We found that IFN-β treatment pushes the expansion of transitional B cells; however, the functionality of transitional B cells in MS is currently unknown. To address this, we assessed the relative amount of IL-10 expressed in transitional B cells (CD19⁺CD24⁺⁺⁺CD38⁺⁺) compared with naive (CD19⁺CD24⁺⁺⁺CD38⁻⁻) and memory B cells (CD19⁺CD38⁻⁻) from five IFN-β–treated MS patients and five healthy volunteers by intracellular FACS (see Materials and Methods) (Fig. 2A). We found that the transitional B cells produced significantly more IL-10 compared with naive B cells in both IFN-β–treated MS patients and healthy volunteers (Fig. 2A, 2B). In addition, we found that the transitional B cells produced more IL-10 compared with memory B cells, although these results did not reach statistical significance. These data demonstrate that the IL-10–dependent

**FIGURE 2.** IL-10 expression in B cell subsets. (A) Representative analysis of intracellular IL-10 staining in transitional (CD19⁺CD24⁺⁺⁺CD38⁺⁺), naive (CD19⁺CD24⁺⁺⁺CD38⁻⁻), and memory (CD19⁺CD38⁻⁻) B cells. (B) Frequency of IL-10⁺⁺⁺ cells in transitional, naive, and memory B cells from five IFN-β–treated MS patients and five healthy volunteers. Statistical significance was determined by one-way ANOVA. (C) IFN-β (1000 U/ml) increases IL-10 secretion in PBMCs from healthy volunteers stimulated with anti-Ig, anti-CD40, and CpG (B-stim). IL-10 was measured in culture supernatants by ELISA, and statistical significance was determined by a Student t test. Error bars represent SEM.
The regulatory function of transitional B cells is intact in IFN-β–treated MS patients.

We next determined the effect IFN-β has on PBMCs from healthy volunteers cultured in the presence or absence of B cell stimulatory conditions. We found that PBMCs stimulated with IFN-β alone had no effect on the secretion of IL-10. Strikingly, when PBMCs were stimulated with anti-Ig, anti-CD40L, and CpG, conditions that stimulate B cell activity (B-stim), IFN-β significantly increased the secretion of IL-10 (Fig. 2C). Because these experiments were performed on whole PBMC cultures, it remains to be determined whether IFN-β acts directly or indirectly on B cells to increase IL-10 secretion. We are actively pursuing this avenue of research. Nonetheless, these data clearly demonstrate that the combination of IFN-β and B cell stimulation induces a potent IL-10 response.

**IFN-β treatment of EAE alters B cell subsets toward a regulatory transitional phenotype**

Because IFN-β increased transitional B cells and plasmablasts in RRMS, we next assessed the effect IFN-β treatment had on B cells in EAE. We treated mice with recombinant mouse IFN-β (10,000 U/dose) or vehicle every second day beginning at EAE day 6 and sacrificed mice for analysis on EAE days 13–16. We found that IFN-β treatment increased levels of BAFF in serum, measured by ELISA, and increased the absolute number of CD19+ B cells in the spleens of WT EAE mice (Fig. 3A, 3B). This increase in B cell numbers was associated with successful treatment (Fig. 3I). We next assessed the specific B cell subsets affected by treatment using flow cytometry. Specifically, we measured the number of transitional (T1 and T2), marginal zone, follicular, plasma cells, and CD11c+CD5+ regulatory B cells in the spleen (Supplemental Fig. 2). We observed that EAE mice treated with IFN-β had significantly higher absolute numbers of splenic T2 B cells, IgD and IgD+ marginal zone B cells, and follicular B cells compared with mice treated with vehicle (Fig. 3C–E). There was a trend of elevated T1 B cells and splenic plasma cells in the mice treated with IFN-β compared with vehicle treated (Fig. 3F, 3G). In addition, we found that the regulatory B cell subset of CD19+CD23hiCD5+ CD1d+IgMhi B cells was significantly increased in the spleens from IFN-β–treated mice compared with vehicle-treated mice (Fig. 3F). Splenic CD19+CD23hiCD5+CD1D+IgMhi B cells share many features with the B1a B cell subset and have been recently described as a regulatory B cell subset (12–14). We also found an elevated percentage of newly formed transitional B cells (AA4.1+ CD19+) in the peripheral blood of WT EAE treated with IFN-β compared with WT EAE treated with vehicle (Fig. 3H). This increase in B cell numbers after therapy was also associated with reduced disease. We found an inverse correlation between EAE severity and the frequency of CD19+ B cells in the spleens, draining lymph nodes, and bone marrows of mice (Fig. 3I and Supplemental Fig. 3). B cells may regulate inflammation in secondary lymphoid tissue or locally within the inflamed CNS tissue. We

![Image](http://www.jimmunol.org/)

**FIGURE 3.** IFN-β treatment alters B cell populations in mice with EAE. Mice with EAE were treated with IFN-β (10,000 U/dose) or vehicle every other day starting at day 6 and throughout the experiment. Mice were sacrificed for analysis at days 13–16 after EAE induction. (A) IFN-β treatment increases BAFF levels in serum from mice with EAE. Serum BAFF concentrations were determined at EAE day 16 in IFN-β (n = 5) or vehicle (n = 5) treated mice by ELISA. Error bars represent SEM, and two-tailed Student t tests were used to determine significance (**p < 0.01). (B) IFN-β treatment increases splenic B cell numbers in mice with EAE. Absolute B cell numbers were determined at EAE day 16 in mice receiving IFN-β (n = 6) or vehicle (n = 6) treatment mice by manual hemocytometer counts and FACS analysis. Error bars represent SEM, and two-tailed Student t tests were used to determine statistical significance (**p < 0.01). (C-G) IFN-β alters B cell subsets in spleens from mice with EAE. Absolute numbers of (C) CD19+IgMhiIgD−CD21−CD23+ transitional 1 and CD19+IgMhiIgD−CD21−CD23+ transitional 2 B cells, (D) CD19+IgD−CD21hi and CD19+IgD+IgMhiCD21hi marginal zone B cells, (E) CD19+IgD−CD21hiIgMhi follicular B cells, (F) CD19+CD23hiCD5+CD1D+IgMhi regulatory B cells, and (G) CD138+B220+CD19hiCD26+CD38+ plasma cells were assessed in spleens from EAE mice treated with IFN-β (n = 3) or vehicle (n = 3) by flow cytometry. Error bars represent SEM, and two-tailed Student t tests were used to determine statistical significance (**p < 0.05, **p < 0.01). (H) IFN-β elevates transitional B cells is confirmed by measurement of AA4.1+ CD19+ B cells in peripheral blood from EAE mice. Representative FACS plots of blood cells from EAE mice treated with IFN-β or vehicle at EAE day 16. (I) Peripheral B cell numbers are inversely correlated with EAE severity despite MOG autoantibody production. Negative correlation between EAE scores and the percentage of CD19+ cells in the spleens of EAE mice. Using linear regression, we determined the slope was nonzero with the indicated R² and the statistical significance was indicated as a p value. Data are representative of IFN-β–treated and vehicle-treated mice (n = 10 total) from two pooled experiments.
mediated CNS injury is unknown. To address this question direct B cells is incidental, promotes inflammation, or regulates cell immunity (2, 10, 12, 14, 16–18). Yet, whether the effect IFN- and there are now several reports of regulatory B cells in autoimmunity (2, 10, 12, 14, 16–18). Yet, whether the effect IFN- and muMT (10,000 U/dose) or vehicle every other day starting at day 6 until day 20 (treatment initiation and cessation are indicated by arrows). WT Veh: n = 15, WT IFN-; n = 14, muMT Veh: n = 6, muMT IFN-; n = 6. Error bars represent SEM, and statistics were determined by a Mann–Whitney U test (*p < 0.05). These data are combined from three independent experiments. (C) Histology of spinal cords isolated from WT and muMT mice treated with IFN- or vehicle. Mice were sacrificed at EAE day 13, and tissue was harvested and stained with both H&E and Luxol fast blue (original magnification ×4). (D) Spinal cord infiltrating CD4+ T cells and CD19+ B cells were assessed by flow cytometry. WT Veh: n = 6, WT IFN-; n = 5, muMT Veh: n = 3, muMT IFN-; n = 3. Statistical significance was determined by one-way ANOVA. (E) IFN- treatment elevates IL-10 in spleen cells from wild type mice. Spleen cells from WT and muMT mice treated with IFN- or vehicle were cultured with 1 µg/ml MOG35–55 for 72 h, and IL-10 was measured in the culture supernatants by ELISA. Statistics were determined by one-way ANOVA.

found no significant differences in the number of B cells in the spinal cords of IFN-– or vehicle–treated EAE mice (Fig. 4D), which suggests that B cells regulate the immune response in the secondary lymphoid tissues and not the CNS.

Effect of IFN- treatment on the development of autoreactive Abs

Because IFN- increased BAFF and altered B cell populations in both RRMS and EAE, we next assessed the effects this treatment had on the humoral immune response in EAE. We observed that IFN- treatment of EAE significantly increased the concentration of the anti-MOG IgG, but not IgM, compared with vehicle-treated EAE (Fig. 5A). However, unlike what we observed with B cell numbers, the increased anti-MOG IgG did not correlate with severity of the disease and occurred despite therapeutic benefit (Fig. 5B). These data provide evidence that the inflammatory injury to the CNS is not mediated by autoantibodies in EAE and also are congruent with the observation that mice deficient in cytidine deaminase (15), an enzyme required for B cell class switching and IgG production, still develop severe EAE comparable with WT animals.

IFN- treatment in EAE requires B cell regulation

IFN- signaling directly and indirectly stimulates B cell survival, and there are now several reports of regulatory B cells in autoimmunity (2, 10, 12, 14, 16–18). Yet, whether the effect IFN- has on B cells is incidental, promotes inflammation, or regulates cell-mediated CNS injury is unknown. To address this question directly, we compared IFN- treatment of EAE induced in both WT and muMT mice, which are unable to produce surface IgM and have an arrest in the maturation of B cells in the bone marrow (19). In contrast to WT mice (Fig. 4A), we found that treatment with IFN- was unable to reduce disease severity in the muMT (Fig. 4B). Histopathology and FACS analysis confirmed the clinical scores (Fig. 4C, 4D). IFN- treatment reduced numbers of infiltrating CD4+ T cells (Fig. 4D) and reduced the number and size of inflammatory lesions in the spinal cords of WT mice (Fig. 4C). In contrast, muMT mice had no observable reduction in the CD4 cell infiltration and lesions load when treated with IFN- (Fig. 4C, 4D). Finally, we found that 1 µg/ml MOG peptide stimulation induced spleen cells from IFN-–treated WT mice (EAE score, 0) to produce significantly more IL-10 than both vehicle–treated WT mice (EAE score, 3) and muMT mice treated with IFN- (EAE score, 3) or vehicle (EAE score, 3; Fig. 4E). These data provide strong evidence that IFN- treatment of EAE requires regulatory B cell function.

IFN- treatment in EAE maintains a population of transient regulatory B cells

Recent reports demonstrate that transfer of terminally differentiated, Ag-experienced regulatory B cells can inhibit EAE and other mouse autoimmunity on adoptive transfer; however, the CD19+ CD23+CD5+CD1D+IgM+B cell has a transient regulatory function and differentiates to plasma cells when transferred into recipient mice (12, 17, 20). To address whether IFN- treatment induces a transient or a terminally differentiated regulatory B cell
MOG Abs and EAE score is also displayed. Rmice (Fig. 6). These data demonstrate that IFN-
tory function.

Furthermore, we directly show that IFN-
B cells that requires BAFF signaling for their development (27).

treatment increases serum BAFF levels and maintains high num-
control mice (\(n = 8\), vehicle (\(n = 8\), or healthy
and IgG by sandwich ELISAs. Two-tailed Student \(t\) tests
were used to determine statistical significance (\(* p < 0.05,
**p < 0.01\)). (B) Linear regression between serum anti-
MOG Abs and EAE score is also displayed. \(R^2\) and
values are displayed. These data are representative of
two pooled experiments.

subset, we isolated B220\(^+\) B cells from the spleens from WT EAE
mice treated with IFN-\(\beta\) or vehicle, and transferred \(20 \times 10^6\) of
these B cells into treatment-naive EAE mice 6 d after immunization
and tracked the development of paralysis. We found that the
progression of EAE did not differ in mice receiving either PBS,
B cells from IFN-\(\beta\)-treated mice, or B cells from vehicle-treated
mice (Fig. 6). These data demonstrate that IFN-\(\beta\) treatment does
not induce a terminally differentiated regulatory B cell, but rather
maintains a population of transitional B cells that have a regula-
tory function.

Discussion
Understanding the mechanisms behind effective IFN-\(\beta\) therapy of
RRMS is an active area of research. Reports have demonstrated
that IFN-\(\beta\) alters chemokine production by myeloid cells to inhibit
EAE, and more recently it has been shown that IFN-\(\beta\) treatment
also generates a novel regulatory T cell subset to inhibit disease
(7, 8). In contrast with RRMS, type I IFNs have an opposing role
in other autoimmune diseases, such as SLE and NMO. In SLE,
endogenous type I IFN drives disease flares, and in NMO IFN-\(\beta\)
treatment induces relapses. This paradoxical role of type I IFN in
RRMS compared with SLE and NMO is also mirrored by the
function BAFF and APRIL play in these diseases. Benlysta, an
anti-BAFF Ab, and high-dose atacicept, a TACI-Ig fusion protein
that blocks BAFF and APRIL, have both been effective in re-
ducing SLE flares (21, 22). This is in contrast with RRMS, where
atacicept worsened disease activity in RRMS patients (23).

Our data, taken in the context of other reports, suggest a thera-
peutic mechanism of IFN-\(\beta\) that bridges the opposing effects
IFN-\(\beta\) and BAFF blockers have in RRMS. Recently, it has been
shown that IFN-\(\beta\) treatment induces the expression of BAFF in
myeloid cells from RRMS patients (4, 24–26). Strikingly, myeloid
cells require type I IFN signaling to attenuate EAE (8). In this
article, we show in both RRMS patients and EAE mice that IFN-\(\beta\)
treatment increases serum BAFF levels and maintains high num-
bers of transient regulatory/transitional B cells, a population of
B cells that requires BAFF signaling for their development (27).
Furthermore, we directly show that IFN-\(\beta\) treatment of EAE
requires B cells to attenuate cell-mediated CNS injury. Therefore,
we propose the following mechanistic model. Treatment with
IFN-\(\beta\) induces BAFF expression in myeloid cells, which in turn
promotes the expansion of transitional B cells. These transitional
B cells have a regulatory function in autoimmune diseases and are
capable of producing large amounts of IL-10 to suppress Ag-
mediated T cell activity in healthy individuals. Remarkably, in
SLE transitional B cells induce effector T cell responses and are
likely proinflammatory (10). Taken together, these data suggest
that in RRMS patients, IFN-\(\beta\) increases BAFF expression, and
unlike in SLE and NMO, skews the B cell population toward
a regulatory phenotype.

Anti-CD20 therapy, such as rituximab, is remarkably effective in
RRMS. This may seem to contradict our model. Yet, rituximab
therapy has been shown to elevate BAFF levels in patients, and
regulatory transitional B cells are preferentially expanded after
anti-CD20 therapy (28), similar to what we found in IFN-
\(\beta\)-treated RRMS, suggesting a possibly convergent mechanism. We
hypothesize that a subset of RRMS patients who do not respond
positively to IFN-\(\beta\) therapy has pathogenic B cell responses or
inadequate activation of regulatory B cell activity. Future studies

FIGURE 5. (A) IFN-\(\beta\) treatment increases serum anti-
MOG IgM and IgG autoantibodies in EAE, although these
Abs do not correlate with EAE severity. Serum from EAE
\(t\)reatment increases serum BAFF levels and maintains high num-
values are displayed. These data are representative of
two pooled experiments.
should evaluate how serum markers of B cell activation during IFN-β therapy correlate with treatment outcome. Although our exploratory clinical data meet statistical significance, we present a small sample size of patients and control subjects. To validate our findings, we are in the process of preparing a larger, longitudinal cohort study.

The work presented in this article complements a growing body of literature on the complexity of B cells in human autoimmunity and provides compelling evidence that IFN-β therapy stimulates a regulatory B cell in RRMS. This has implications for the monitoring and treatment of RRMS patients treated with IFN-β. From the standpoint of discovery-based research, future work should evaluate more directly the contribution BAFF and APRIL have on the therapeutic effect of IFN-β.

Acknowledgments

We thank the Oklahoma Medical Research Foundation imaging core for the immunohistochemistry.

Disclosures

R.D.S., J.D., and R.C.A. have filed a provisional patent based on the data presented in this manuscript.

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