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Glatiramer acetate immune modulates B-cell antigen presentation in treatment of MS

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Abstract

Objective

We examined the effect of glatiramer acetate (GA) on B-cell maturation, differentiation, and antigen presentation in MS and experimental autoimmune encephalomyelitis (EAE).

Methods

A cross-sectional study of blood samples from 20 GA-treated and 18 untreated patients with MS was performed by flow cytometry; 6 GA-treated patients with MS were analyzed longitudinally. GA-mediated effects on B-cell antigen-presenting function were investigated in EAE, or, alternatively, B cells were treated with GA in vitro using vehicle as a control.

Results

In MS, GA diminished transitional B-cell and plasmablast frequency, downregulated CD69, CD25, and CD95 expression, and decreased TNF-α production, whereas IL-10 secretion and MHC Class II expression were increased. In EAE, we observed an equivalent dampening of proinflammatory B-cell properties and an enhanced expression of MHC Class II. When used as antigen-presenting cells for activation of naive T cells, GA-treated B cells promoted development of regulatory T cells, whereas proinflammatory T-cell differentiation was diminished.

Conclusions

GA immune modulates B-cell function in EAE and MS and efficiently interferes with pathogenic B cell–T cell interaction.

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Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

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Glossary

APC = antigen-presenting cell; **EAE** = experimental autoimmune encephalomyelitis; **GA** = glatiramer acetate; **NMO** = neuromyelitis optica; **PBMC** = peripheral blood mononuclear cell; **PBS** = phosphate-buffered saline; **RRMS** = relapsing-remitting MS; **WT** = wild type.

Glatiramer acetate (GA), a synthetic random basic copolymer composed of glutamic acid, lysine, tyrosine, and alanine, is widely used in the treatment of MS.[1] GA has been shown to reduce relapse rates and progression of neurologic disability.[2] The precise mechanism of action by which GA mediates this benefit is still not fully understood. Studies showed a preferential differentiation of $CD4^+$ T cells into T helper (Th)-2 cells,[3,4] downregulation of Th17 cell differentiation,[5] increased frequency and function of $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells,[6,7] and modulation of $CD8^+$ T cells.[8] Moreover, GA was found to promote M2 monocyte differentiation[7,9] and to reduce activation and proinflammatory cytokine secretion in monocytes[9,10] and plasmacytoid dendritic cells.[11]

Several lines of evidence highlight essential roles of B cells in the pathogenesis of MS.[12,13] This is broadly supported by the beneficial effect of B cell–depleting therapies, both in relapsing-remitting (RR)MS[14,15] and primary progressive MS.[16,17] Some studies have also shown immunomodulatory properties of GA on B cells, including reduction in the number of circulating B cells and a shift from a proinflammatory to an anti-inflammatory B-cell phenotype.[18–20] The functional consequences of these phenotypical changes yet remained to be elucidated. Accordingly, we here focused on the question to what extent GA-mediated effects on B cells can change their ability to act as antigen-presenting cells (APCs) for the activation of T cells, an assumed key process in the development and propagation of MS.

Methods

Human

Blood samples were collected from patients with relapsing-remitting MS at the Clinical MS Center of the Universitätsmedizin Göttingen (UMG) in Germany between 2015 and 2018. The diagnosis of RRMS was based on the McDonald criteria. Twenty patients with MS naive to approved disease-modifying therapy were treated with GA for ≥1 month. Eighteen untreated patients with MS served as controls. Six GA-treated patients were analyzed longitudinally, having had blood samples taken at 2 different time points with an interval of at least 3 months. Demographic and disease-related information is summarized in the table.

Mice

Six- to ten-week-old female wild-type (WT) C57BL/6 mice were purchased from Charles River. MOG p35-55 TCR transgenic 2D2 mice were kindly provided by Dr. Kuchroo (Boston, MA).

Ethical approvals

Ethical approvals for all human samples used were given by the ethical review committee of the UMG (approval number 27/4/14). All animal experiments were performed in accordance with the guidelines of the Central Department for Animal Experiments, UMG, and approved by the Office for Consumer Protection and Food Safety of the State of Lower Saxony (protocol number 33.9-42502-04-17/2615).

EAE induction and scoring

Female WT mice were immunized with 50 μg MOG peptide$_{35-55}$ MEVGWYRSPFSRVVHLYRNGK emulsified in complete Freund's adjuvant followed by intraperitoneal injections of 100 ng of *Bordetella pertussis* toxin at the day of immunization and 2 days thereafter. Experimental autoimmune encephalomyelitis (EAE) severity was assessed daily and scored on a scale from 0 to 5 scale as described.[21]

GA treatment

GA was provided by Teva Pharmaceutical Industries. Mice received daily SC injections of 150 μg GA suspended in 0.1 mL phosphate-buffered saline (PBS) or PBS alone.

Detection of anti-GA antibodies

Ninety-six-well plates were coated with 10 μg/mL GA in PBS overnight. Thereafter, diluted serum samples were incubated for 2 hours. After washing, plate-bound antibodies were detected with horseradish peroxidase–conjugated anti-mouse IgG, directed against the Fc part of the bound antibodies. Absorbance was measured at 450 nm with subtraction of a 540-nm reference wavelength on the iMark Microplate Reader.

Isolation of human and murine leukocytes

Human immune cell counts were determined in our hospital's routine laboratory. Human peripheral blood mononuclear cells (PBMCs) were isolated after Ficoll gradient centrifugation and cryopreserved at −80°. Splenic B cells were purified by negative magnetic activated cell sorting (MACS) separation using a mouse lineage panel. Splenic T cells were isolated by negative MACS separation using a mouse pan T-cell isolation kit II.

Flow cytometry

Pregating and gating strategy for human B-cell subsets and surface molecule expression was done as described in figure e-1 (links.lww.com/NXI/A218). Fc receptors were blocked using human TruStain FcX. Dead cells were stained with a fixable viability kit. Human B-cell differentiation was determined using CD19 (HIB19), CD20 (L27), CD24 (ML5), CD27 (O323), and CD38 (HIT2). B-cell activation was evaluated by CD25 (BC96), CD40 (5C3), CD69 (FN50), CD80 (L307.4), CD86 (FUN-1), CD95

Table Patient characteristics

	Untreated	GA treated (GA1)	GA treated (GA2)
No. of patients	18	20	6
Age (y) (mean ± SD)	32.4 ± 9.7	42.0 ± 9.4	42.6 ± 9.8
Female sex (%)	77.7	54.5	50.0
EDSS score (mean ± SD)	2.1 ± 1.7	2.0 ± 1.4	2.4 ± 1.3
Disease duration (y) (mean ± SD)	4.7 ± 6.2	7.4 ± 6.1	9.8 ± 5
GA since (mo) (mean ± SD)	—	5.9 ± 4.1	6.5 ± 5.6

Abbreviation: GA = glatiramer acetate.

(DX2), and major histocompatibility complex (MHC) II (Tü36) after 2 μg/mL CpG stimulation for 20 hours. T cells and monocytes were determined using CD4 (RPA-T4), CD8 (HIT-8a), and CD14 (M5E2), respectively. To investigate B-cell cytokine production, cell suspensions were stimulated with 1 μg/mL CpG, 500 ng/mL ionomycin and 20 ng/mL phorbol 12-myristate 13-acetate in the presence of 1 μL/mL brefeldin A for 22 hours followed by cell fixation/permeabilization and intracellular staining for IL-6 (MQ2-13A5), IL-10 (JES3-19F1), and TNF-α (MAb11). For murine experiments, Fc receptors were blocked using monoclonal antibody specific for CD16/CD32 (93). Dead cells were stained with a fixable viability kit. Splenic B-cell activation/differentiation was determined using CD19 (6D5), CD25 (PC61), CD69 (H1.2F3), CD40 (3/23), CD80 (16-10A1), CD86 (GL-1), and MHCII (AF6-120.1) after 1 μg/mL CpG stimulation for 20 hours. To investigate B-cell cytokine production, cell suspensions were stimulated with 1 μg/mL CpG in the presence of 1 μL/mL brefeldin A for 6 hours followed by cell fixation/permeabilization and intracellular staining for IL-10 (JES5-16E3) and IL-6 (MP5-20F3). Treg cell differentiation was evaluated by CD4 (GK1.5), CD25 (PC61), and by intracellular staining for FoxP3 (FJK-16s) after fixation and permeabilization using the fixation/permeabilization kit. To investigate Th1 and Th17 cell differentiation, cell suspensions were stimulated with 50 ng/mL phorbol 12-myristate 13-acetate and 0.5 μg/mL ionomycin in the presence of 1 μL/mL brefeldin A for 6 hours followed by a CD4 (GK1.5) staining. Cytokine production was analyzed by intracellular staining for IFN-γ (XMG1.2) and IL-17A (TC11-18H10). Samples were acquired on a BD LSRFortessa. All data evaluation was performed using FlowJo software.

ELISA

Production of cytokines was measured using ELISA MAX Standard Set kits. Absorbance was measured at 450 nm with subtraction of a 540-nm reference wavelength on the iMark Microplate Reader.

T-cell proliferation assay

MACS-purified splenic B cells were cocultured with MACS-purified MOG-specific CFSE-stained (CFSE Cell Division Tracker Kit) splenic T cells from 2D2 mice and were restimulated with MOG peptide$_{35\text{-}55}$. After 72 hours, T-cell proliferation was evaluated by flow cytometry.

Statistical analysis

Statistical analysis was performed using the software GraphPad Prism 5.01 and 6.01. Human data sets were tested for Gauss distribution using the D'Agostino-Pearson omnibus normality test, Shapiro-Wilk normality test, and Kolmogorov-Smirnov normality test. For the comparison of 2 cross-sectional cohorts with Gauss distribution an unpaired and for longitudinal samples a paired *t* test was used, respectively. If the data were not Gauss distributed, a Mann-Whitney *U* test was applied in the cross-sectional analysis, and the Wilcoxon matched-pairs signed-rank test was used for the longitudinal data. Clinical scores and T-cell proliferation are depicted as mean ± SEM and were analyzed by the Mann-Whitney *U* test. GA antibody titers are shown as median and were analyzed using the Student *t* test. All other data are shown as median, and the statistical comparison was made using the Mann-Whitney *U* test. A value of $p < 0.05$ was considered significant and is shown by 1 asterisk. Two asterisks and 3 asterisks indicate significances of $p < 0.01$ and $p < 0.001$, respectively.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Results

In the present study, we compared 20 GA-treated patients with MS with 18 untreated MS controls (table). The mean GA therapy duration was 5.9 months and ranged from 1 to 14.5 months. Six GA-treated patients with MS were analyzed longitudinally at 2 different time points with an interval of >3 months. Patients were naive to any approved disease-modifying therapy and had not received steroids for at least 3 months before GA treatment.

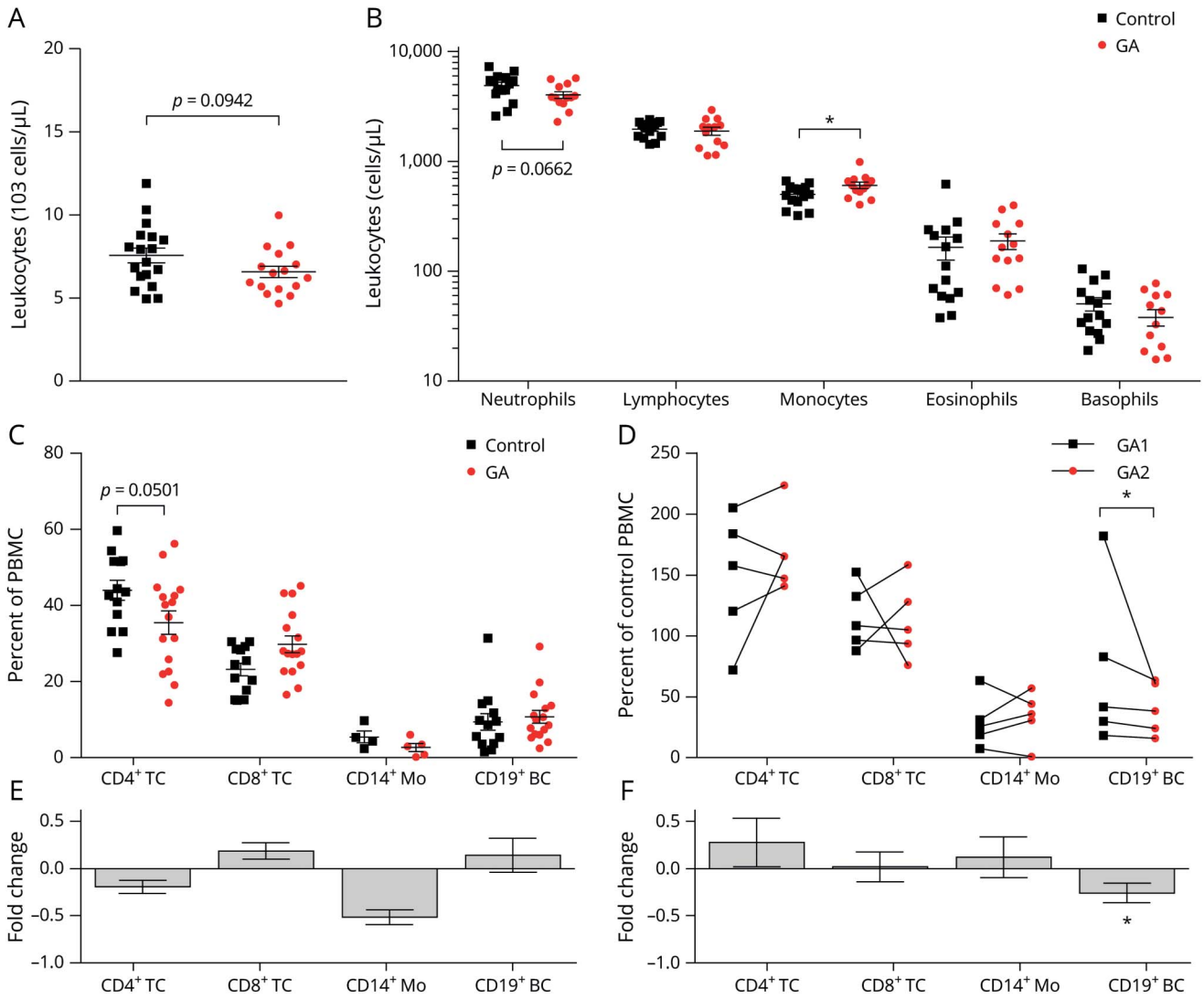
GA alters PBMC composition

At first, we determined the impact of GA on PBMC composition. GA treatment resulted in a trend toward decreased absolute leukocyte and neutrophil numbers, whereas monocyte numbers became significantly elevated (figure 1, A and B). Frequency analysis of the cross-sectional and longitudinal study showed a tendency for decreased $CD4^+$ T cells and significantly diminished $CD19^+$ B cells, respectively (figure 1, C–F). This GA treatment effect did not correlate the respective treatment duration ranging between 1 and 14.5 months (figure e-2A, links.lww.com/NXI/A218).

GA decreases B-cell activation, differentiation, and proinflammatory cytokine production, whereas IL-10 secretion and MHC Class II expression are increased

To assess whether GA has an effect on B-cell activation, differentiation, and cytokine production, we analyzed

Figure 1 GA treatment alters the composition of leukocytes in patients with MS



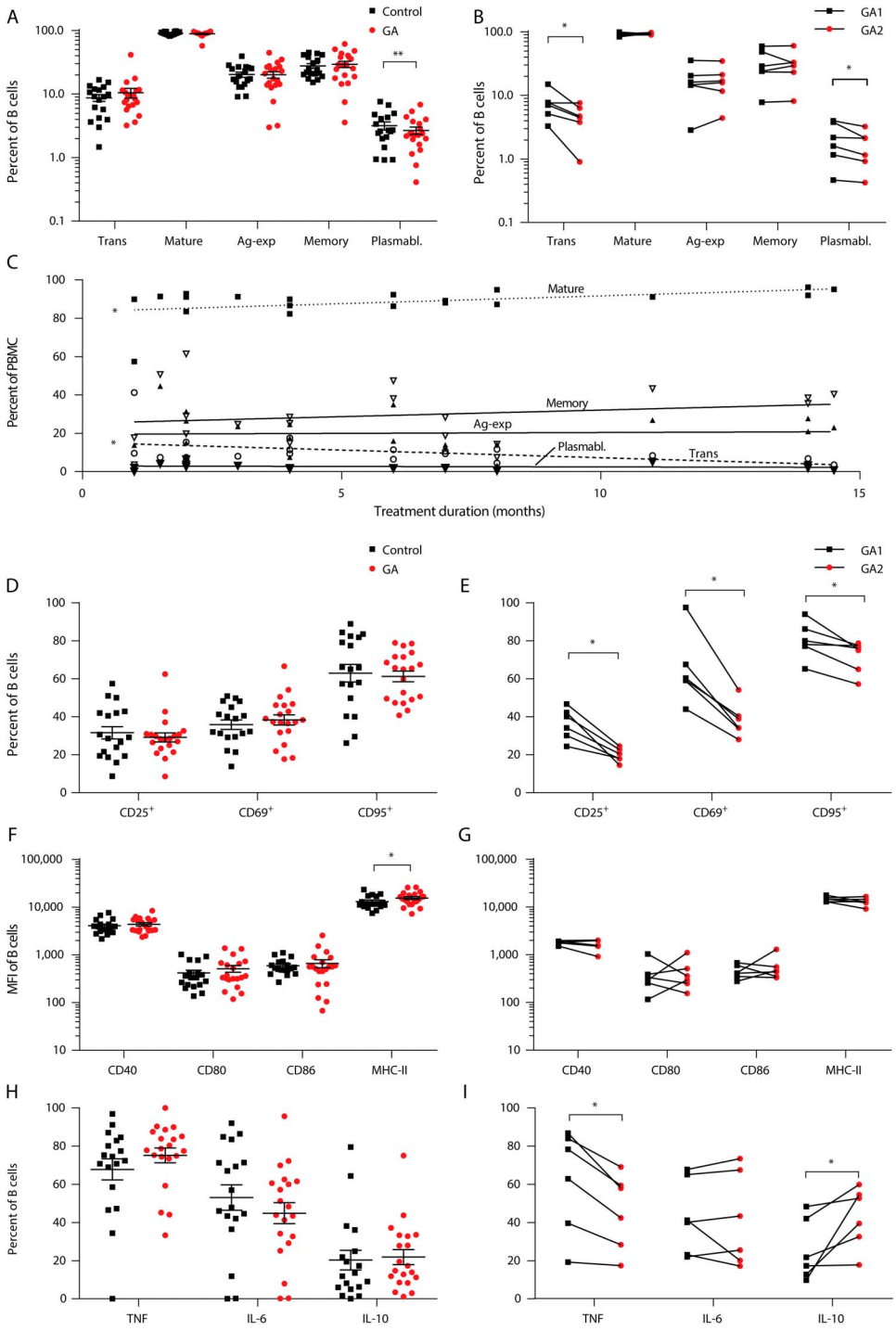
Peripheral blood samples were taken from control (n = 18) and glatiramer acetate–treated (GA; n = 20) patients with MS. (A) Leukocyte counts and (B) neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts were measured in routine clinical laboratory blood counts if available (*$p < 0.05$; unpaired *t* test). Next, peripheral blood mononuclear cells (PBMCs) were isolated from the samples. (C) Cell frequencies of $CD4^+$ T cells (TC), $CD8^+$ TC, $CD14^+$ monocytes (Mo), and $CD19^+$ B cells (BC) were determined using flow cytometry (ns; unpaired *t* test). (D) $CD4^+$, $CD8^+$ TC, $CD14^+$ Mo and BC of patients with MS at 2 time points during GA medication; line connects an individual patient (n = 5; *$p < 0.05$; Wilcoxon matched-pairs signed-rank test). (E and F) Fold changes of the horizontal and longitudinal cell frequency changes. GA = glatiramer acetate.

peripheral blood B cells (figure 2, A–I, figure e-2, B and C, links.lww.com/NXI/A218). GA therapy was associated with a reduction in plasmablasts, both in the cross-sectional and longitudinal analyses of blood samples (figure 2, A and B). In addition, frequency of immature transitional B cells was decreased in the longitudinal study (figure 2B) and correlated with GA treatment duration (figure 2C). GA longitudinally downregulated CD25, CD69, and CD95 expression on B cells, whereas MHC Class II expression was upregulated as compared to untreated MS controls (figure 2, D–G). Other molecules involved in antigen presentation such as CD40, CD80, and CD86 showed no difference (figure 2, F and G). IL-6 production was not altered by GA treatment, both in the cross-sectional and longitudinal studies, whereas GA increased anti-inflammatory IL-10 and decreased proinflammatory TNF-α cytokine production in the longitudinal analysis; however, no correlation with longer GA treatment duration was found (figure 2, H and I, figure e-2B, links.lww.com/NXI/A218).

GA upregulates MHC Class II B-cell expression independent of EAE

To identify whether the observed reduction on B-cell activation, TNF-α production and the increase in IL-10 secretion and MHC Class II expression in patients with MS is a result of GA treatment or a concomitant disease-related effect, we initially administered daily subcutaneous GA to naive unimmunized wild-type mice (figure 3A). GA had no

Figure 2 GA therapy changes the phenotype of human B cells in patients with MS



Human peripheral blood mononuclear cells (PBMCs) were isolated from glatiramer acetate (GA; n = 20) or non-GA (control; n = 18) treated patients with MS. In addition, 6 patients were analyzed longitudinally on GA treatment. Red circles represent GA treatment, squares control treatment. (A) Mean frequency ± SEM of B-cell subpopulations defined as follows: transitional B cells ($CD24^{high}$ $CD38^{high}$; transitional), mature B cells ($CD24^{var}$ $CD38^{low}$; mature), antigen-activated B cells ($CD27^{+}$; ag-activated), memory B cells ($CD27^{var}$ $CD38^{-}$; memory), and plasmablasts ($CD20^{-}$ $CD27^{+}$ $CD38^{+}$; *$p < 0.05$; unpaired *t* test). (B) B-cell subset frequencies of patients with MS at 2 time points during GA therapy; line connects an individual patient (n = 6; *$p < 0.05$; Wilcoxon matched-pairs signed-rank test). (C) The individual patients' frequencies of BC subsets were correlated with the duration of GA treatment (*$p < 0.05$; linear regression). (D) MFI ± SEM of activation molecules expressed on B cells (ns; unpaired *t* test). (E) B-cell activation marker expression of patients with MS at 2 time points during GA therapy; line connects an individual patient (n = 6; *$p < 0.05$; Wilcoxon matched-pairs signed-rank test). (F) Mean MFI of molecules involved in antigen presentation expressed on B cells (*$p < 0.05$; unpaired *t* test). (G) Expression of molecules involved in antigen presentation of patients with MS at 2 time points during GA medication; line connects an individual patient (ns; Wilcoxon matched-pairs signed-rank test). (H) Shown is the frequency of positive cells regarding the respective cytokine (tumor necrosis factor [TNF], interleukin [IL]-6, and IL-10; mean ± SEM; ns; unpaired *t* test). (I) TNF, IL-6, and IL-10-positive B cells of patients with MS at 2 time points during GA medication; line connects an individual patient (*$p < 0.05$; Wilcoxon matched-pairs signed-rank test). GA = glatiramer acetate.

impact on B-cell activation or cytokine production; however, MHC Class II was significantly upregulated after ex vivo stimulation (figure 3, B–H).

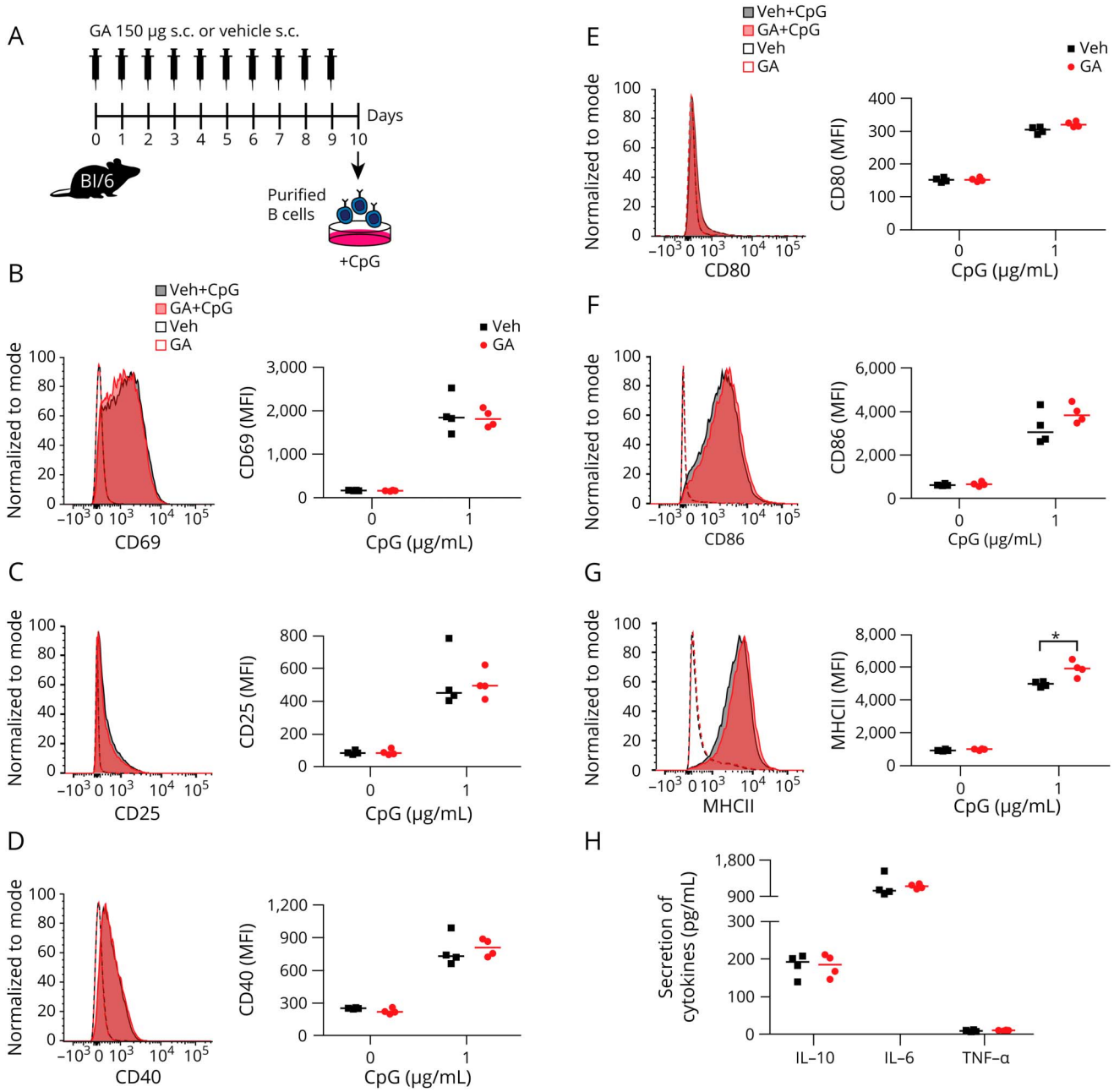
GA downregulates B-cell activation and ameliorates clinical severity of active EAE

To investigate the effect of GA on B-cell phenotype and function during pathologic conditions, mice received a daily subcutaneous GA injection, starting 7 days before immunization (figure 4A). GA ameliorated EAE (figure 4B), which was associated with a production of antibodies against GA (figure 4C), a decrease in expression of the early activation marker CD69 on B cells, and diminished secretion of IL-6, whereas the expression of costimulatory molecule CD86 and MHC Class II was upregulated (figure 4D).

GA increases B-cell antigen-presenting capacity resulting in regulatory T-cell induction

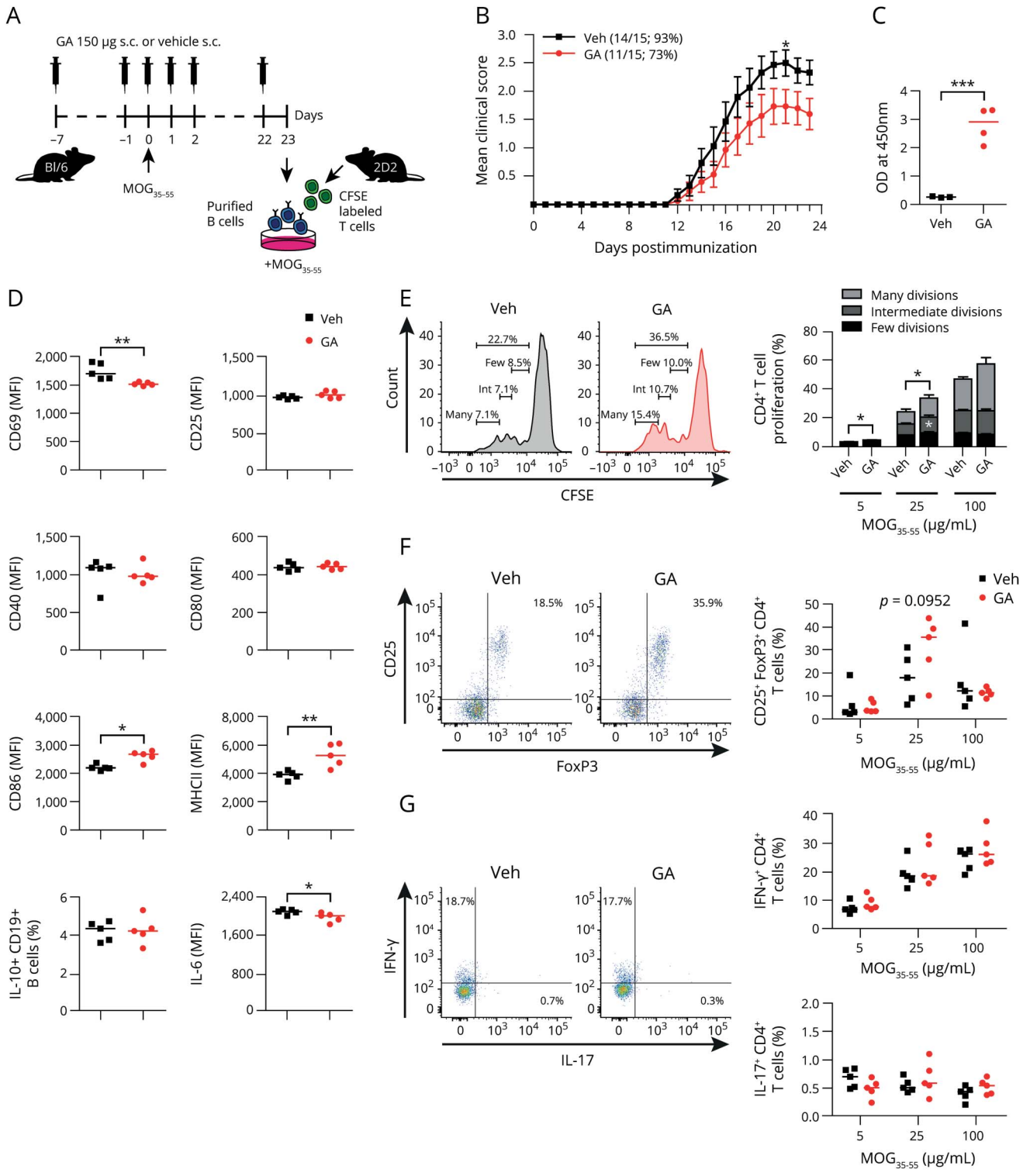
To elucidate whether our findings on B-cell properties have mechanistic consequences on antigen-presenting function,

Figure 3 GA upregulates MHC Class II expression on B cells



(A) Naive mice received a daily SC injection of 150 µg GA. On day 10 post-treatment onset, splenic B cells were isolated and analyzed (B and C) for expression of activation markers, (D–F) costimulatory molecules, and (G) the antigen-presenting molecule MHC Class II as well as (H) for secretion of cytokines. Data are shown as median; n = 4; *$p < 0.05$; Mann-Whitney U test. GA = glatiramer acetate.

Figure 4 GA prevents B-cell activation and ameliorates clinical severity of active EAE



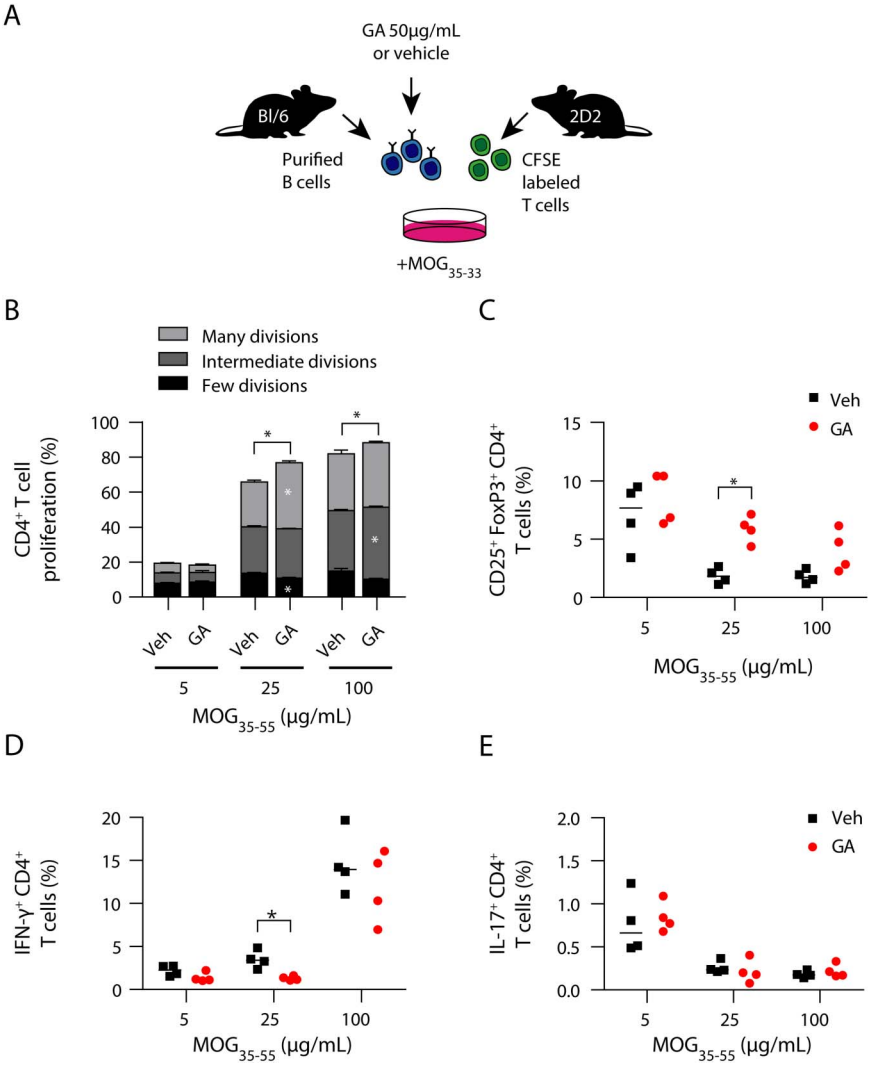
(A) GA therapy was performed by a daily SC injection of 150 µg, starting 7 days before MOG peptide$_{35\text{-}55}$ immunization. Serum and splenic B cells were isolated on day 23 post-immunization. (B) Mean group EAE severity is given as mean ± SEM; disease incidence is indicated in brackets; n = 15; *p < 0.05; Mann-Whitney U test. (C) GA antibody titers were measured at 450 nm (data given as median; n = 3–4; ***p < 0.001; Student t test). (D) B-cell activation, expression of molecules involved in antigen presentation, and cytokine secretion were analyzed by FACS (data given as median; n = 5; *p < 0.05, **p < 0.01; Mann-Whitney U test). (E) B cells were cocultured with CFSE-labeled myelin-specific (2D2) naive T cells in the presence of 5, 25, or 100 µg/mL MOG peptide$_{35\text{-}55}$. T-cell proliferation was evaluated by CFSE dilution and stratified by division frequency as follows: few divisions (1–2; black), intermediate divisions (3; medium gray), and many divisions (≥4; light gray). T-cell divisions are shown as mean ± SEM; n = 5; *p < 0.05; Mann-Whitney U test. Differentiation of myelin-specific naive T cells into (F) Treg cells ($CD25^+FoxP3^+CD4^+$) or (G) Th1- ($IFN\text{-}\gamma^+CD4^+$) and Th17 cells ($IL\text{-}17^+CD4^+$) was analyzed by FACS (data given as median; n = 5). EAE = experimental autoimmune encephalomyelitis; GA = glatiramer acetate.

B cells were isolated 30 days post-GA treatment onset and 23 days post-EAE induction and cocultured with MOG-specific (2D2) T cells in the presence of increasing MOG peptide$_{35\text{-}55}$ concentrations (figure 4A). As shown in figure 4E, B cells purified from GA-treated mice triggered a significantly higher proliferation of myelin-specific T cells. Importantly, this related to an expansion of Treg cells (figure 4F), whereas Th1- and Th17 cell frequencies remained unaffected (figure 4G). Based on these findings, we next assessed the direct effect of GA exposure on B-cell APC function in vitro. Purified naive B cells were pre-incubated with GA following coculture with myelin-specific T cells in the presence of MOG peptide$_{35\text{-}55}$ (figure 5A). GA pre-incubation resulted in a B-cell stimulatory effect (figure e-3, links.lww.com/NXI/A218), which was accompanied by enhanced capacity to generate Treg cells, paralleling our ex vivo findings on GA treatment (figure 5, B–E).

Discussion

GA has been shown to reduce the relapse rate and progression of neurologic disability in MS.[2] Past studies demonstrated anti-inflammatory properties of GA on T cells[4,6,8] and myeloid cells.[9,10,22] First lines of evidence indicate an immunomodulatory effect on B cells,[18–20] although it remained unclear whether this may affect the ability of B cells to act as APCs. In this article, we investigated the phenotype and APC function of B cells in MS and its murine model on treatment with GA. We found decreased frequencies of immature (transitional) B cells and plasmablasts in GA-treated patients with MS. A reduction in circulating CD19$^+$ B cells in GA-treated patients with RRMS has been also described previously,[23] which could reflect diminished B-cell survival factors such as BAFF and APRIL after GA therapy, as it was observed in EAE.[20] In this regard of interest may be that we found a correlation between high baseline B-cell frequencies, an

Figure 5 GA-treated B cells preferentially generate T regs, whereas development of proinflammatory T cells is diminished



(A) Naive B cells purified from WT mice were incubated with 50 µg/mL GA or vehicle at 37°C for 3 hours. After washing, B cells were cocultured with CFSE-labeled myelin-specific (2D2) naive T cells in the presence of 5, 25, or 100 µg/mL MOG peptide$_{35\text{-}55}$. (B) T-cell proliferation was evaluated by CFSE dilution and stratified by division frequency as follows: few divisions (1–2; black), intermediate divisions (3; medium gray), and many divisions (≥4; light gray). T-cell divisions are shown as mean ± SEM; n = 4; *p < 0.05; Mann-Whitney U test. Differentiation of myelin-specific naive T cells into (C) Treg cells (CD25$^+$FoxP3$^+$CD4$^+$) or (D) Th1- (IFN-γ$^+$CD4$^+$) and (E) Th17 cells (IL-17$^+$CD4$^+$) was analyzed by FACS (data given as median; n = 4; *p < 0.05; Mann-Whitney U test). GA = glatiramer acetate.

active disease course, and a poor GA treatment response (figure e-2C, links.lww.com/NXI/A218), possibly suggesting that patients with MS with increased peripheral blood B-cell numbers might not properly respond to GA therapy.

By longitudinally analyzing the GA effect on B-cell phenotype, we observed a downregulation of the activation marker CD69, CD95, and CD25 and a decrease in TNF-α production and an increase in IL-10 secretion, which was supported by a recent study showing a shift toward anti-inflammatory cytokine production by B cells on GA therapy.[19] Of interest, we found a modest but significant upregulation of MHC Class II expression on B cells in GA-treated patients with MS. B cells are thought to act as APCs for presentation of GA to T cells.[24] Direct binding of GA to multiple murine and human MHC Class II epitopes[25,26] has been shown, raising the question whether our observation might have consequences in terms of B-cell APC function. To address this pivotal issue, we first administered GA to naive WT mice to rule out a disease-related effect and indeed noticed an upregulation of MHC Class II expression on B cells, without any effect on other markers of activation. During pathologic conditions following EAE induction, GA treatment decreased clinical severity, B-cell activation, and proinflammatory cytokine production, whereas the costimulatory molecule CD86 and MHC Class II were again upregulated. To further elucidate the observed B-cell immune modulation with focus on B-cell antigen presentation, we used a coculture in which purified B cells from GA-treated mice or alternatively naive B cells following GA preincubation in vitro were used as APCs to activate naive myelin-specific T cells. GA-treated B cells triggered a significantly higher proliferation of naive myelin-specific T cells, composed of increased $CD4^+CD25^+FoxP3^+$ Treg cells. As TGF-ß is associated with the development of Treg cells, we also measured TGF-ß production by B cells in our model, however at no detectable levels. This mechanistic observation, which is well supported by earlier reports on an expansion of Treg cells on GA treatment in MS,[6] and indicates that GA centrally interferes with pathogenic B cell–T cell interaction in development and propagation of CNS demyelinating disease.

Our findings indicate common features to IFN-β, which also have been shown to exert immunomodulatory properties on B cells by abrogating proinflammatory and by fostering anti-inflammatory cytokine production.[27] However, IFN-β is thought to primarily downregulate costimulatory molecules and MHC-Class II,[27–29] our findings suggest the modulation of B-cell antigen presentation by GA as a key role for B cell–fostered Treg cell development.

Anti–CD20-mediated B-cell depletion has been shown to be a very efficient therapy in MS,[14–17] however, treatment cessation may lead to a recovery of highly differentiated pathogenic B cells,[30] and long-term treatment may lower immunoglobulin production, possibly raising the risk of infections over time.[31] Our data support the concept that GA could act as a suitable maintenance therapy after cessation of anti-CD20 treatment by fostering regulatory properties in repopulating B cells. The first trial in humans provided inconclusive results.[32] Although the beneficial effect by GA as maintenance therapy showed superior efficacy than GA therapy alone, this benefit seemed to wane within the study period. More trials are needed, as that study was limited due to a small number of patients and the lack of a control group receiving no maintenance therapy after rituximab cessation.

Moreover, GA could also have beneficial effects in other B cell–mediated diseases such as neuromyelitis optica (NMO). Although aquaporin-4 antibody (AQP4-IgG)-seropositive patients showed inefficient results,[33,34] first lines of evidence indicate that patients with AQP4-IgG–seronegative NMO may respond to GA therapy.[33,35,36]

In conclusion, our data indicate that the pleotropic immunomodulatory effect of GA includes B cells and B-cell antigen presentation resulting in a normalization of MS-specific pathogenic B-cell differentiation and in an expansion of Treg cells. These novel findings may complement other established effects of GA in MS, may pioneer its preferential use after B-cell depletion, and may lastly be of clinical relevance in other B cell–driven CNS autoimmune diseases.

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Disclosure

D. Häusler, Z. Hajiyeva, J.W. Traub, S.S. Zamvil, P.H. Lalive, and W. Brück report no disclosures. M.S. Weber is serving as an editor for *PLoS One*. Go to Neurology.org/NN for full disclosures.

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Appendix Authors

Name	Location	Contribution
Darius Häusler, PhD	UMG	Performed mouse experiments and analyzed the data; prepared the figures; and wrote the manuscript
Zivar Hajiyeva, MD	UMG	Performed human experiments and analyzed the data and wrote the manuscript
Jan W. Traub, MD	UMG	Prepared the figures and reviewing and editing
Scott S. Zamvil, MD, PhD	University of California, San Francisco	Reviewing and editing
Patrice H. Lalive, MD	University of Geneva	Reviewing and editing
Wolfgang Brück, MD	UMG	Reviewing and editing
Martin S. Weber, MD	UMG	Supervised the research and wrote the manuscript

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