were collected every 2 d, and ELISAs detecting total IgM and total IgG were performed. Data from five mice per group were shown. *p , 0.05, **p , 0.01.

FIGURE 5. CD1d^{high} CD5^{+} Bregs are resistant to MEDI551 depletion. LN cells were isolated at peak of the disease (days 14–16) from MEDI551 or control Ab (250 mg)-treated mice and assessed by immunofluorescence staining followed by flow cytometry analysis. (A) Representative plot of FACS analysis of the CD1d^{high} CD5^{+} Bregs in the LN. Numbers represent the relative frequencies of CD1d^{high} CD5^{+} Bregs within mouse CD19^{+} gates. (B) Bar graphs indicate mean (6 SEM) percentages of CD1d^{high} CD5^{+} cells in mouse CD19^{+} gates as shown in (A). (C) Bar graphs indicate mean (6 SEM) absolute cell number of CD1d^{high} CD5^{+} cells in mouse CD19^{+} gates as shown in (A) and (B). (D) Ratio of cell numbers of Bregs and non-Breg B cells (other CD19^{+} B cells) in the LNs were bar-graphed in the MEDI551-treated compared with control Ab-treated mice. Data from five to six mice per group are shown and are representative of two independent experiments. **p , 0.01.

FIGURE 6. MEDI551 day 7 treatment inhibits the induction of MOG-specific Th17 and Th1 cells but promotes expansion of MOG-specific Foxp3^{+} Tregs. Bulk draining LN cells harvested at peak of the disease (days 14–16) were CFSE labeled and stimulated with 30 mg/ml rhMOG in culture. Ninety-six hours later, MOG-specific T cells were analyzed by intracellular staining and flow cytometry analysis, and supernatants were harvested for cytokine measurements. (A) Representative histograms and (B) bar graphs show the proliferation of rhMOG-responsive CD4 and CD8 T cells determined by CFSE dilution. (C and D) Quantification of IFN-g and IL-17A secretion in the culture supernatants by Bioplex. (E) CD4^{+}CFSE^{low} populations were further gated based on the expression of Foxp3, IFN-g, and IL-17A. (F) Bar graphs show the summarized data of frequencies of MOG-specific Th1 (IFN-g^{+}) cells, Th17 (IL-17A^{+}) cells, and Tregs (Foxp3^{+}) in the CD4^{+}CFSE^{low} populations. Error bars indicate SEM. Data from six mice per group are shown and are rep- resentative of three independent experiments. *p , 0.05, **p , 0.01, ***p , 0.001, ****p , 0.0001.

Supplemental Figure 1. Gating strategies used in this study. A-C) 10-color survey panel to identify leukocyte subsets in the CNS. At peak of the disease, brain and spinal cord mononuclear cells were isolated from PBS perfused animals and subjected to FACS analysis. Data shown is the FACS analysis of MNC isolated from the spinal cord of control Ab-treated mice. A) Cells were first gated on FSC-A and SSC-A to identify mononuclear cells (MNC). After singlet gating, cells were gated on yellow viability dye to define viable cells. B) Neutrophils (CD11b^{+}Gr1^{+}), activated monocyte/microglia (CD11b^{+}Gr1^{-}), NK cell (NK1.1^{+}CD3e^{-}) and lymphocytes (CD45^{hi}CD11b^{-}) were identified in the viable cell population. C) Lymphocytes were further subdivided based on the expression of CD3e, TCRβ, CD4, B220 and mouse CD19. T cells were identified as CD3e^{+}

and further divided to αβT cells ($TCR\beta^+$) and γδ T cells ($TCR\beta^-$). CD4 and CD8 T cells were identified in αβT cell population based on the presence or absence of CD4 expression. $CD19^+$ B cells were identified in the $CD3e^-$ fraction. Of note is that the population negative for CD19 but positive for B220 which may represent plasmacytoid dendritic cells (pDC) was excluded from the B cell gate. D) Analysis of CD19 and CD20 expression on CD138+ plasma cells. Splenocytes from EAE mice at the peak of disease were stained with antibodies to detect human CD19 and mouse CD20 expression on $CD138^+$ plasma cells ($IgD^-CD3^-CD138^+B220^-$) and IgD^+ B cells ($IgD^+mCD19^+B220^+$). Data are representative of two independent experiments.

Supplemental Figure 3. B cell depletion by MEDI551 *in vivo*. hCD19Tg mice immunized with $rhMOG_{1\text{-}125}$ were i.p. injected with 250μg MEDI551 or Control Ab on day 7 post- immunization. A) Blood were taken at indicated time points and subjected to FACS analysis for monitoring circulating $CD19^+$ B cells (n=8). B) At peak of the disease (day 14-16), cells from bone marrow, spleen, lymph node, peritoneal cavity, brain and spinal cord were harvested for FACS analysis. Cells were stained with anti-CD45, anti- B220 and anti-mouse CD19, and then examined by flow cytometry. Upper panel: Numbers indicate the percentage of $CD19^+$ B cells in $CD45^+$ lymphocyte gates. Bottom panel: Bar graphs indicate the mean (±SEM) percentage of $CD19^+$ B cells within $CD45^+$ lymphocyte gates (n=4). Data are representative of three independent experiments.

Supplemental Figure 4. Detection of cytokine-secreting T cells and Th17-related cytokines in MEDI551 day7 treated mice. Mice were sacrificed at peak of the disease and subjected to various assays. A-C) Frequency of each cytokine-producing T cell subset in $CD4^+$ T populations from the spinal cord (A), brain (B) and LN (C). D-G) Cytokines including IFN-γ, IL-17A, IL-1β, TNF-α, IL-10 and IL-6 were quantified by Bioplex. D) Spinal cord supernatants; E) brain supernatants; F) serum; G) Culture supernatants of LN cells in the recall assay shown in Figure 6.

Data from five to six mice per group are shown. Data are representative of three independent experiments.

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