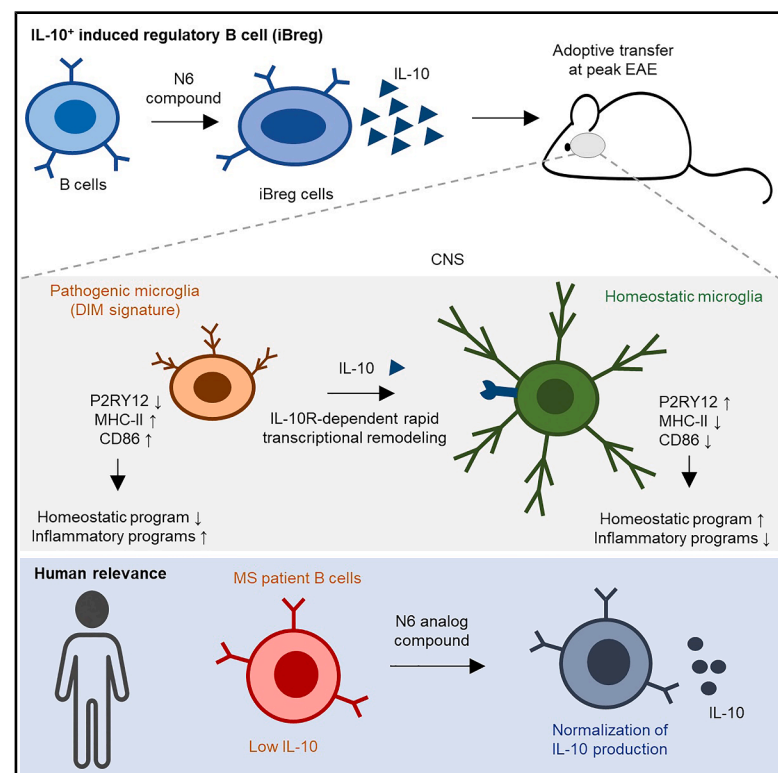


Induced regulatory B cells stably expressing interleukin-10 reverse central nervous system autoimmunity by targeting myeloid cells

Graphical abstract



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In brief

Interleukin (IL)-10 produced by B cells has regulatory functions in disease contexts. Manfroi et al. report a strategy for the efficient generation of IL-10-producing B cells using a chemical compound identified through a high-throughput screen. Upon transfer, these IL-10⁺ B cells reverse ongoing CNS autoimmunity at disease peak in a manner dependent on IL-10 receptor expression in myeloid cells.

Highlights

- B cells stably producing IL-10 can be efficiently generated using chemical compounds
- Induced regulatory B cells reverse CNS autoimmunity upon transfer at disease peak
- IL-10 receptor signaling in long-lived CX3CR1⁺ cells is required for disease reversion
- IL-10 expression can be normalized in B cells from MS patients using chemical compounds

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Article

Induced regulatory B cells stably expressing interleukin-10 reverse central nervous system autoimmunity by targeting myeloid cells

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SUMMARY

The lack of approaches to produce interleukin (IL)-10-producing B cells at high frequency has hampered mechanistic investigation into the generation, fate, and activity of these regulatory cells. We screened pharmacological compounds for their ability to induce IL-10 expression in B cells. One compound, N6, promoted stable IL-10 expression in >95% of B cells. Transfer of IL-10⁺ B cells induced remission of experimental autoimmune encephalomyelitis. N6-induced B cells accumulated in the central nervous system (CNS) and differentiated into IL-10⁺ plasma cells, promoting transcriptional and phenotypic changes in myeloid cells within the CNS. The therapeutic effect of IL-10⁺ B cell transfer was abrogated in mice lacking IL-10 receptor expression in long-lived myeloid cells, pointing to microglia as targets of B cell-derived IL-10. Importantly, an N6 analog corrected defective IL-10 expression in B cells from multiple sclerosis patients. Our findings establish a pharmacological strategy to generate IL-10⁺ B cells with therapeutic potential and identify microglia as critical targets for intercepting neuroinflammatory disease progression.

INTRODUCTION

The immune system has evolved to provide defenses against infectious diseases. Besides infections, antimicrobial immune re-

sponses can also cause tissue pathology. It follows that the activation of the immune system is often associated with tissue pathology. As a counteracting process, regulatory immune cells provide mechanisms to control ongoing inflammation and initiate

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tissue repair programs. Elucidating the regulatory pathways active specifically during ongoing immune responses may inform therapeutic strategies for chronic immune-mediated inflammatory diseases (IMIDs).^{1,2}

Upon activation, B cells can express interleukin (IL)-10 and exert regulatory functions in ongoing autoimmune, allergic, infectious, and malignant diseases, as well as in transplantation in mice and humans.^{3–7} B cell regulatory functions involve the canonical signaling pathways implicated in B cell activation, such as intrinsic signaling via the B cell receptor (BCR), CD40, or Toll-like receptors (TLRs).^{3,8} In multiple sclerosis (MS), B cells often exhibit impaired IL-10 production,⁹ and normalized IL-10 expression in a patient subgroup correlates with improved disease course.¹⁰ A defect in IL-10 expression in B cells might thus facilitate disease progression in this human disease. In the pre-clinical model for MS experimental autoimmune encephalomyelitis (EAE), mice with an *Il10* deficiency restricted to B cells develop a severe chronic disease, whereas mice with wild-type (WT) B cells spontaneously recover after a short paralysis.³ IL-10-producing B cells can be used as cell therapy to intercept disease.^{3,11–13}

Although the utilization of IL-10-producing B lymphocytes in cell therapy is appealing, this is currently challenged by the lack of strategy to produce such therapeutic cells. The lack of such a strategy also limits the investigation of the mechanisms underpinning their differentiation from naive B cells and the elucidation of the processes behind their capacity to reverse clinical signs during ongoing inflammatory diseases.

The mechanisms controlling the resolution versus chronification of ongoing immune-mediated disease indeed remain incompletely characterized even though CD4⁺ T cells and myeloid cells are known to be involved.^{14–17} Thus, the contribution of specific myeloid cell types such as infiltrating monocytes, resident macrophages, and microglia, as well as the signals competent for their control have not yet been fully delineated with specific genetic approaches. The establishment of an approach to intercept ongoing disease without depleting any cell type would facilitate

the analysis of the cellular and molecular circuits driving disease progression versus those driving its resolution.

We aimed to establish a strategy to easily produce high numbers of IL-10-producing B cells (iBreg cells) and to examine their therapeutic capacity. A high-throughput screen identified IL-10-inducing compounds allowing the production of B cell cultures in which >95% of cells stably expressed IL-10. iBreg cells reversed ongoing EAE in recipient mice upon adoptive cell therapy, which operated independently of their reactivity toward a disease-relevant autoantigen through the modulation of CNS monocytes, macrophages, and microglia.

RESULTS

Identification of chemical compounds allowing the production of B cells highly enriched in IL-10 expression

TLR and CD40 signaling are important for the regulatory functions of B cells.^{3,8} However, these signals induced *Il10* in fewer than 30% of B cells *in vitro* (Figure S1A). To improve this response, we established a high-throughput screen to evaluate chemical libraries by flow cytometry. B cells from *Il10*.EGFP reporter mice were stimulated with individual compounds from a 40,128-molecule library and lipopolysaccharides (LPSs, TLR4 agonist) for 3 days. The frequency of *Il10*-expressing cells was then analyzed by flow cytometry. This screen and validation steps (see [high-throughput flow cytometry screen](#) section in [method details](#)) led to compound C3 as inducer of *Il10* expression in B cells (Figure S1A). Because TLR9 agonists can activate both mouse and human B cells¹⁸ while LPS poorly stimulates human B cells, we then focused on C-phosphate-G (CpG) oligonucleotides. The assessment of 9 chemical analogs to C3 on B cells stimulated via TLR9 identified N6 as more efficient compound (Figure 1A). C3 and N6 were active at low concentration (250 nM) (Figure 1A) and across various stimulation protocols (Figure S1A). N6 augmented the frequency of *Il10*-producing cells from ~24% to ~75% in B cells activated with CpG for 3 days (Figures 1A and S1A), without affecting viability

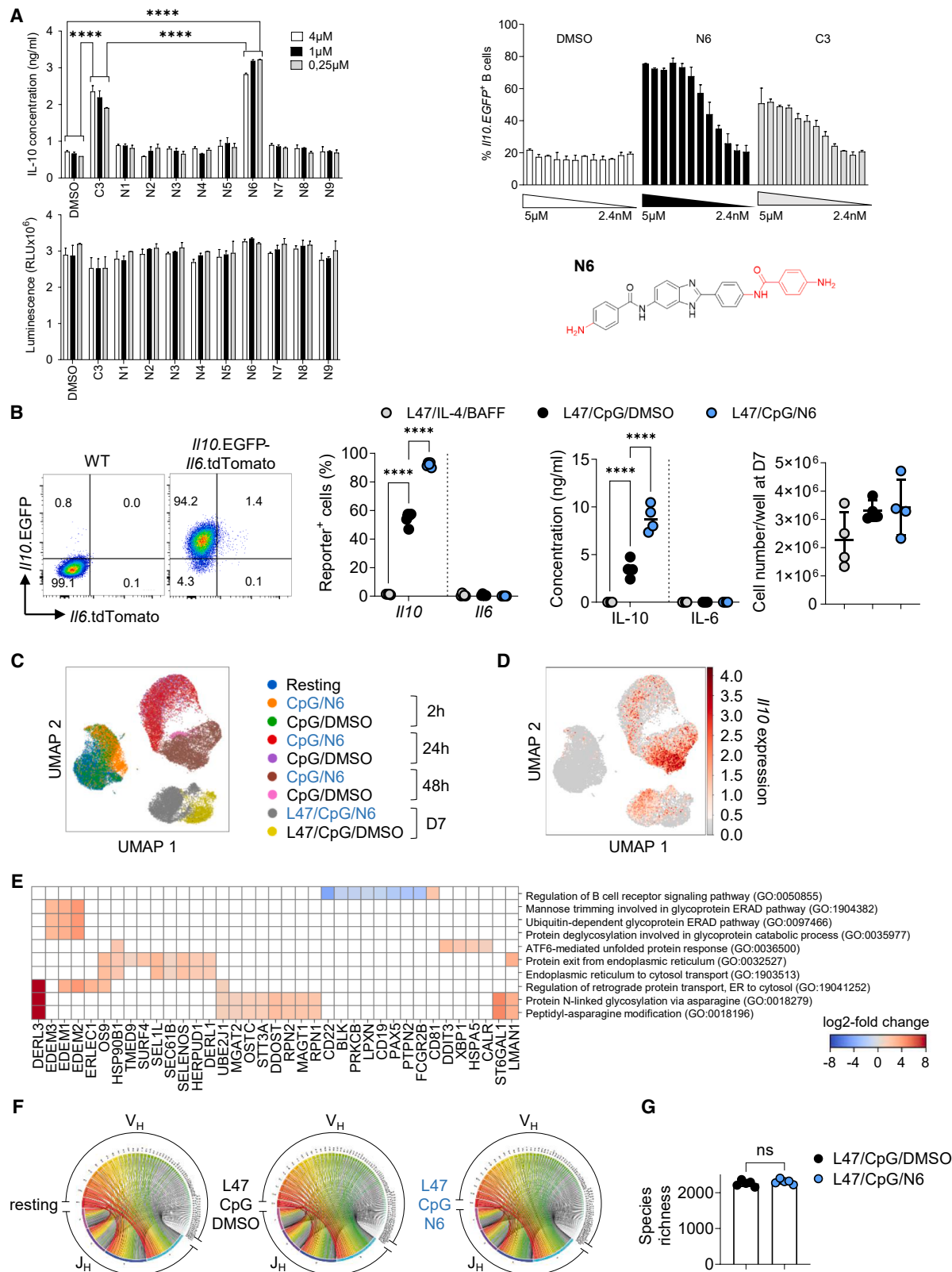


Figure 1. Ex vivo generation of highly enriched I10-expressing plasmacytes

(A) Left: mouse WT B cells mice were stimulated with CpG and C3 or similar molecules and supernatants analyzed by Bio-Plex (mean $\pm$ SEM, $n = 2$ independent experiments) (top). Cell viability was analyzed using a CellTiter-Glo assay (bottom) (mean $\pm$ SD, $n = 2$ independent experiments). Right: B cells from I10. EGFP-

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(Figure 1A) and expression of IL-6 (data not shown), a mediator of pathogenic B cell functions in autoimmune diseases.^{19,20}

To increase the impact of N6, the 3-day culture was extended by 4 days in presence of N6, CpG, and CD40L-expressing L47 cells. This 2-step protocol yielded highly enriched (~95%) *Il10*-expressing B cells and amplified the B cell number by 30-fold (Figure 1B). Control cultures with vehicle (DMSO) had only ~50% *Il10*-expressing cells (Figure 1B). B cells re-stimulated with L47/IL-4/B cell activating factor (BAFF) during the second culture step did not express *Il10* (Figure 1B). B cells did not express IL-6 in any of these 7-day cultures (Figure 1B). Similar results were obtained using an agonistic antibody to CD40 instead of L47 cells (Figure S1B). The up-regulation of *Il10* expression similarly occurred when IL-10 and IL-10 receptor (IL-10R) were blocked (Figure S1C). This protocol fully polarized splenic follicular (FO) B cells and marginal zone (MZ) B cells into *Il10* producers (Figure S1D), even though FO B cells have been reported to be less proficient at expressing IL-10.^{5,21}

To characterize the effect of N6, B cells activated for 2 h, 24 h, 48 h, and 7 days with CpG and N6 or DMSO vehicle, as well as resting B cells, were analyzed by single-cell RNA sequencing (scRNA-seq, 10× Genomics). A uniform manifold approximation and projection (UMAP) of these data showed the impact of N6 on B cells along their activation (Figure 1C). *Il10* became detectable at 24 h and was preferentially localized in N6-B cell areas (Figures 1C and 1D). A Gene Ontology (GO) search on day 7 samples revealed an enrichment for genes associated with the unfolded protein response (UPR) in N6-B cells (Figure 1E), which was confirmed by projecting the inferred UPR pathway activity on the UMAP (Figure S1E). This suggested that N6 promoted B cell differentiation into plasmocytes.²² Indeed, compared with control cultures, N6-B cells displayed a higher frequency of B220^{lo}CD138^{hi} plasmocytes (Figure S1F) and a higher expression of *PR domain zinc finger protein 1* (*Prdm1*) (Figure S1G) that encodes B lymphocyte-induced maturation protein 1 (BLIMP1), a master transcription factor for plasmocyte differentiation. This enhanced plasmocyte differentiation was preserved when IL-10R signaling was blocked (Figure S1H). We next assessed whether *Il10* expression in B cells was mechanistically linked to their differentiation into plasmocytes. To this end, *Prdm1* and *interferon regulatory factor 4* (*Irf4*) genes were inactivated in B cells using CRISPR-Cas9 before they were exposed to

N6. In brief, B cells from *H11.Cas9-Il10.EGFP-Il6.tdTomato* were pre-activated at day -1 with anti-CD180 for 24 h, then transduced at day 0 with retroviral vectors to express the guide RNA (gRNA), a puromycin resistance gene, and a blue fluorescent protein (Figure S2A). B cells were then immediately subjected to the 7-day protocol. B cell stimulation with anti-CD180 led to their activation (thus enabling their retroviral transduction), without inducing their differentiation into *Il10*- or *Il6*-expressing cells (Figure S2B). As expected, the inactivation of either *Irf4* or *Prdm1* prevented plasmocyte differentiation (Figure S2C). The inactivation of *Prdm1* did not affect *Il10* expression, while *Irf4* inactivation reduced the frequency of *Il10*-expressing cells from around 91% to 69% (Figure S2C). Plasmocyte differentiation is thus not strictly required for *Il10* expression, even though the two processes involve *Irf4*.

We next examined whether N6 induced a bias in BCR repertoire. There was no obvious bias in V_H-J_H repertoire between N6-B cells and DMSO-control B cells at day 7 (Figures 1F and 1G).

Collectively, this work established a strategy permitting, with the identified compounds, the production of large numbers of B cells showing a high proportion of IL-10-producing cells.

N6 induces stable IL-10 expression in treated B cells

To identify the effects of N6 in B cells that were constant along the culture period, we searched for genes stably modulated in B cells by N6. Comparing the single-cell transcriptomes of N6-treated and control B cells at 2 h, 24 h, 48 h, and day 7 post-activation and the bulk transcriptomes of unstimulated B cells exposed to N6 or DMSO for 6 h, we found that *Il10* and nineteen other genes were differentially expressed in at least 4 of these 5 comparisons between N6-treated samples and controls (*Arhgap6*, *Armxc6*, *Cd36*, *Cd81*, *Edem3*, *Ehd4*, *Ets2*, *Hepacam2*, *Ifngr1*, *Il7r*, *Klra4*, *Leprot1*, *Net1*, *P2ry14*, *Phgdh*, *Rnd3*, *Saraf*, *St3gal5*, and *Stim2*). The projection of this “N6 gene signature” on the UMAP (Figure S3A) and of its genes in a heatmap (Figure S3B) illustrated its higher expression in N6-B cells.

To gain further insights into the stability of N6's effects, we analyzed the epigenome of N6-treated and control B cells using assay for transposase-accessible chromatin using sequencing (ATAC-seq). A principal component analysis (PCA) of ATAC-seq data highlighted the distinct epigenetic profiles of N6-treated

Il6.tdTomato mice were stimulated with CpG for 3 days, with N6 or C3 (2-fold dilutions starting at 5 μ M) or DMSO as control (mean $\pm$ SD, n = 2 independent experiments) (top). N6 structure is shown (bottom).

(B) B cells from WT and *Il10.EGFP-Il6.tdTomato* mice were stimulated with CpG/N6 for 3 days and then transferred on CD40L-expressing cells (L47) with IL-4/BAFF, CpG/DMSO (DMSO as vehicle control), or CpG/N6 (N6 initially dissolved in DMSO) for 4 days. Representative fluorescence-activated cell sorting (FACS) plots for *Il10.EGFP* and *Il6.tdTomato* expression at day 7 for cultures with L47/CpG/N6 for the last 4 days (left) and quantification on viable/singlets/CD45⁺CD19⁺ cells (middle) (mean $\pm$ SD, n = 5 independent experiments). B cells from WT mice were used to determine autofluorescence. Similar cultures were repeated with WT B cells to analyze supernatants by ELISA for IL-10 and IL-6 at day 7 and to determine B cell number at day 7 (mean $\pm$ SD, n = 4 independent experiments). (C) A UMAP was generated by integrating the scRNA-seq data obtained from samples stimulated as indicated.

(D) *Il10* expression was projected on the UMAP.

(E) A GO enrichment was performed on differentially expressed genes between L47/CpG/DMSO- and L47/CpG/N6-generated cells (day 7). Genes expressed at higher level in N6-treated cells are in red in the log₂-fold change scale.

(F) Mouse WT B cells were stimulated as in (B), and immunoglobulin heavy-chain repertoire sequencing was performed. Circos plots depicting the frequencies of V_H-J_H usage and combinations of productive sequences are shown (representative of 5 independent cultures).

(G) Species richness of 3,000 randomly selected variable-diversity-joining (VDJ) reads (mean $\pm$ SD from 5 independent cultures).

Statistical analyses were performed with a two-way ANOVA followed by Tukey's multiple comparisons test (A and B) or with an unpaired Mann-Whitney test (G). ns > 0.05, **** p < 0.0001.

See also Figures S1 and S2.

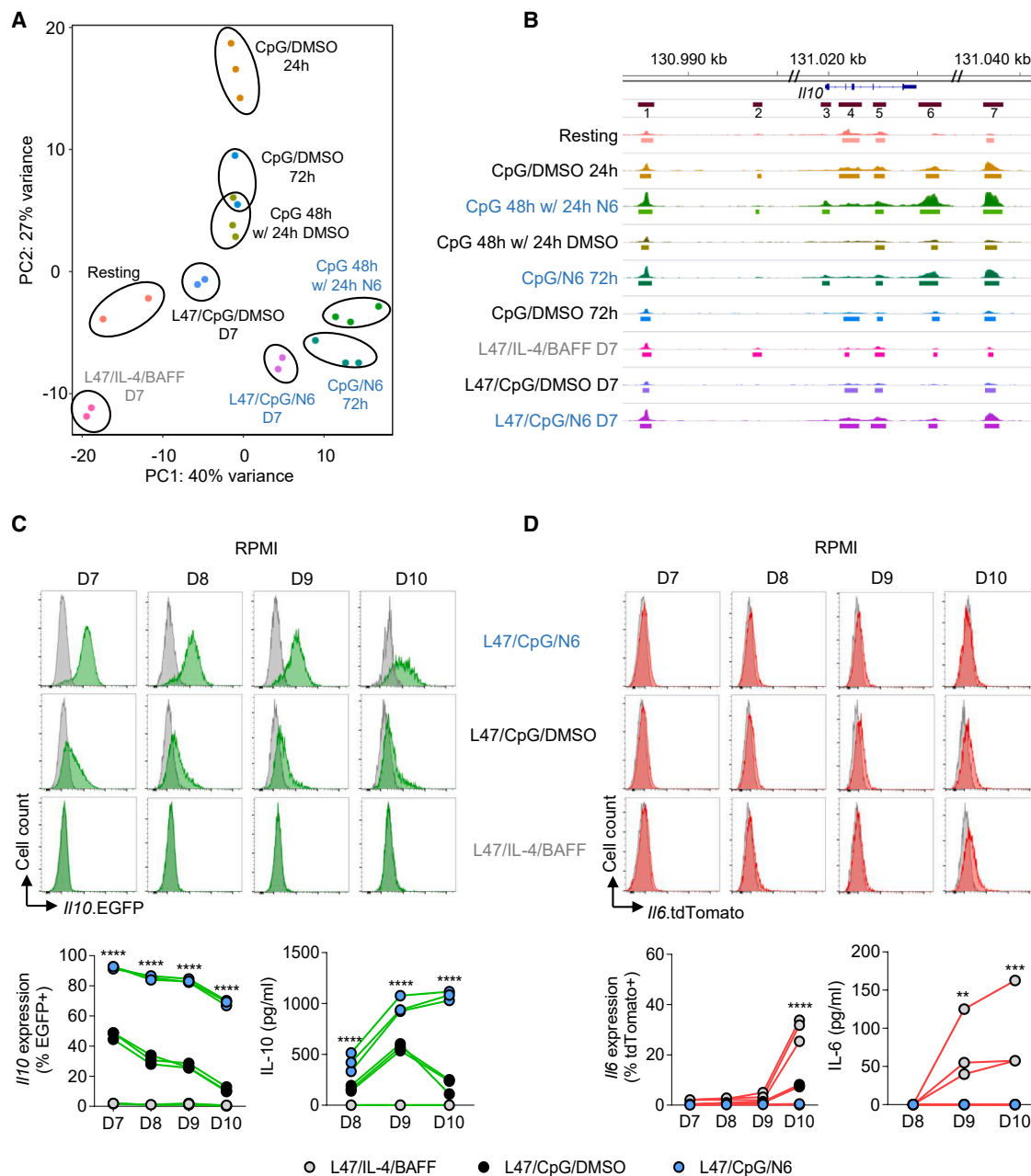


Figure 2. N6 confers IL-10 expression stability in B cells

(A) Mouse B cells were stimulated as indicated and analyzed by ATAC-seq. Data are shown as principal component analysis (PCA).

(B) DARs from all pairwise comparisons indicated by 7 brown rectangles and model-based analysis for ChIP-seq 2 (MACS2) ATAC peaks showing accessible regions of the *Il10* region.

(C and D) Cells generated as in Figure 1B were washed and left unstimulated (RPMI) for 3 additional days and analyzed daily for *Il10* (C) and *Il6* (D) expression. B cells from WT and *Il10*.EGFP-*Il6*.tdTomato reporter mice were used to determine autofluorescence and *Il10* or *Il6*, respectively. Representative histograms are shown (top). Gray and green or red histograms represent autofluorescence and *Il10* or *Il6*, respectively. Quantification is shown (bottom left). Supernatants from WT B cells were analyzed by ELISA (bottom right). Data pooled from 3 independent experiments.

Statistical analyses were performed with a two-way ANOVA followed by Tukey's multiple comparisons test (C and D). ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. See also Figure S3 and Table S1.

and control B cells (Figure 2A). N6-treated B cells tended to group together and separately from the control groups regardless of the culture duration, suggesting a specific and stable epigenetic reprogramming. N6 increased *Il10* locus accessibility in B cells

within 24 h (cells stimulated with CpG for 24 h and then CpG + N6 for 24 h; CpG 48 h w/ 24 h N6 in Figures 2A and 2B) acting at the promoter and several enhancers (Figure 2B). At this time point, N6 increased accessibility at 1,264 differentially accessible

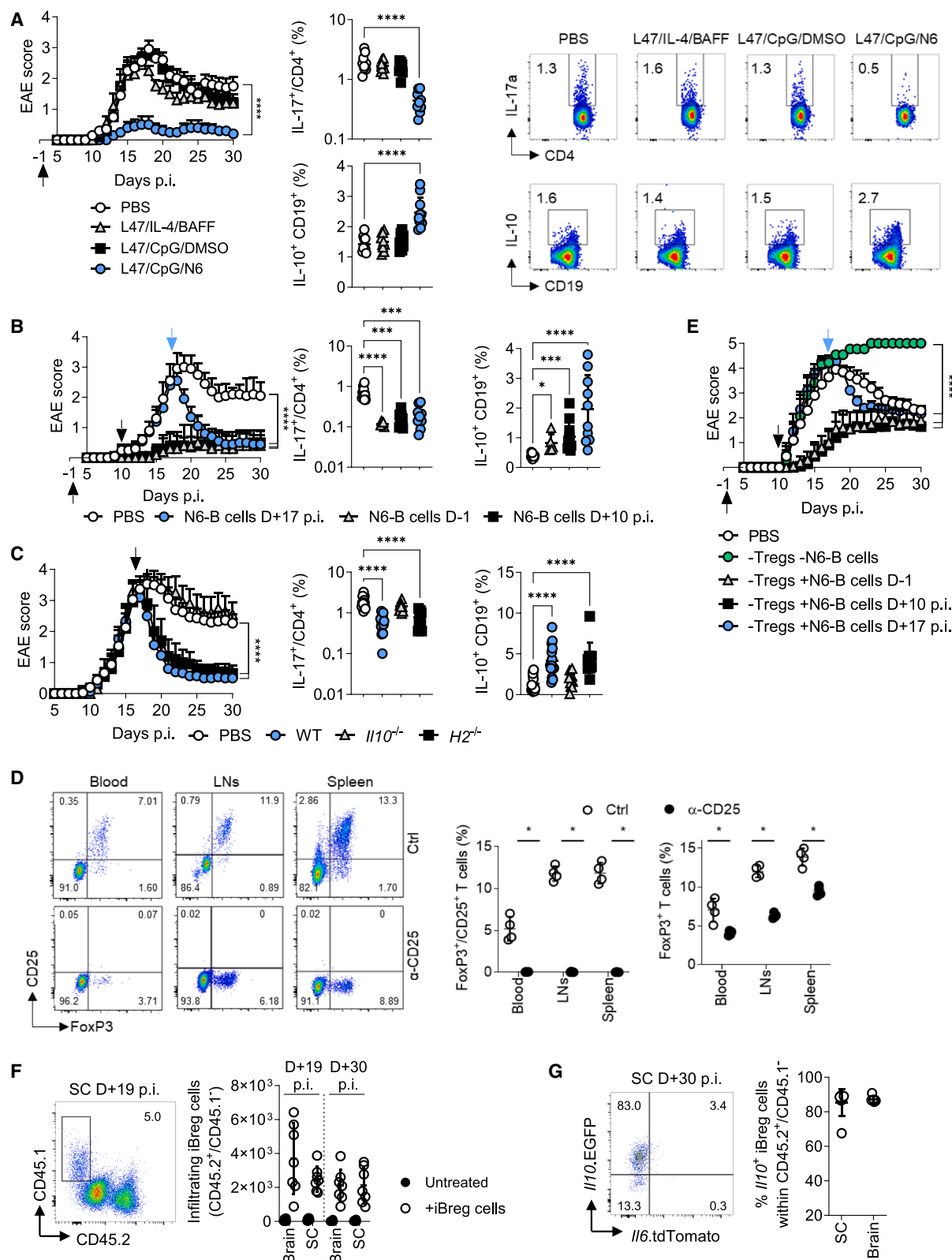


Figure 3. iBreg cells protect mice from EAE

(A) EAE was induced in WT mice and WT B cells stimulated as in Figure 1B were administered on day -1. Data show clinical scores (mean ± SEM, *n* ≥ 9 mice/group, 2 independent experiments). At day +30 p.i., splenocytes were stimulated for 5 h with MOG₃₅₋₅₅ and analyzed by flow cytometry for frequency of IL-17⁺ in

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regions (DARs) and reduced it at 46 DARs, indicating that only a limited number of regions were affected, mainly in introns, intergenic regions, and promoters (Table S1). Several genes, including *Il10*, had multiple DARs (Table S1). The accessibility at these 1,310 DARs remained globally distinct in N6-B cells across time, as indicated by their separation from control samples in the unsupervised clustering (Figure S3C). The accessibility of the 20 genes composing the “N6 gene signature” was then analyzed. Among these genes *Il10*, *Rnd3*, and *Stim2* had DARs showing an increased accessibility in N6-B cells at all tested time points (Figure S3D).

The durable increase in *Il10* expression and in genomic accessibility at the *Il10* locus achieved by N6 suggested that N6 might induce stable IL-10-producing B cells. To test this possibility, B cells were cultured for 7 days as described in Figure 1B, washed, and then re-cultured in different conditions for 3 days without N6. N6-B cells displayed a stable *Il10* expression from days 8 to 10, when kept in medium (RPMI, Figure 2C) or when re-activated with CpG (Figure S3E). Similar results were obtained when N6-B cells were re-cultured with an anti-CD40 and/or anti-immunoglobulin M (IgM), with CpG, LPS, or R848 (Figure S3E; data not shown). Accordingly, the amount of IL-10 increased from days 8 to 9 and, depending on the condition, increased or remained stable from days 9 to 10 (Figures 2C and S3E; data not shown). In contrast, control B cells displayed a progressive loss of *Il10* expression from days 8 to 10 and reduced IL-10 in supernatants on day 10 compared with day 9 in all conditions (Figures 2C and S3E; data not shown). N6-B cells did not express *Il6* between days 8 and 10 unlike control B cells stimulated with L47/CpG/DMSO or L47/IL-4/BAFF from days 4 to 7 (Figures 2D and S3F; data not shown).

We conclude that N6 induces highly enriched and stable IL-10-producing B cells.

Therapeutic effect of induced IL-10-producing B cells against CNS autoimmunity

We next determined the protective effect of “N6-B cells” (corresponding below to B cells activated with CpG/N6 for 3 days followed by L47/CpG/N6 for 4 days, as in Figure 1B) in EAE. 10^7 N6-B cells were administered once to recipient mice 1 day before

immunization with myelin oligodendrocyte glycoprotein peptide (MOG_{35–55}) peptide. This almost completely suppressed EAE development in recipient mice, whereas B cells generated with L47/IL-4/BAFF or L47/CpG/DMSO during the second culture step had no effect on disease course (Figure 3A). Protection was associated with a reduced CD4⁺ autoreactive T_H17 response and an increased accumulation of IL-10⁺ B cells (Figure 3A). N6-B cells had no effect on the frequency of CD25⁺FoxP3⁺ Treg cells, CD4⁺ T cells expressing lymphocyte-activation gene 3 protein (LAG-3) and/or programmed cell death 1 (PD-1), autoreactive CD4⁺ T cells expressing granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), interferon γ (IFN- γ), IL-10, or CD40L (Figures S4A and S4B). It also had no effect on the relative frequency of B cell subsets (FO, MZ, double-negative [DN]) (Figure S4C). N6-B cells similarly controlled EAE development when administered at day +10 post-immunization (p.i.) (Figure 3B).

Remarkably, a single administration of 10^7 N6-B cells into recipient mice near the peak of EAE led to rapid disease resolution, whereas PBS-treated controls maintained a persistent paralysis (Figure 3B). As demonstrated by histology, treated mice showed lower numbers of infiltrating CD3⁺ T cells as well as Mac-3⁺ myeloid cells, an improved myelination status, and a preserved neuronal integrity in spinal cords at day +30 p.i. (Figure S4D). Serum neurofilament light chain (sNfL), a biomarker of neuronal damage and disease activity in MS patients,²³ was strongly increased at day +17 p.i. (data not shown) and N6-B cell treatment triggered a higher reduction of sNfL levels at day +30 p.i. compared with untreated mice (Figure S4D). The immune phenotyping of mice treated at days +10 or +17 p.i. with N6-B cells highlighted the reduction of CD4⁺ autoreactive T_H17 cells and the increased frequency of IL-10-producing B cells as key distinctive features (Figure 3B), while other parameters analyzed, such as frequencies of Treg cells, CD4⁺ T cells expressing LAG-3 and/or PD-1, autoreactive CD4⁺ T cells expressing GM-CSF, TNF, IFN- γ , IL-10, or CD40L, or the proportion of FO, MZ, and DN cells within B cells, were unchanged (Table S2; Figures S4E–S4G). The clinical and immunological effects of N6-B cells depended on their expression of IL-10 but were independent of major histocompatibility complex class II

CD4⁺ T cells. At day +30 p.i., splenocytes were also stimulated for 5 h with PMA/ionomycin/LPS and analyzed for frequency of CD19⁺IL-10⁺ B cells. Data show quantifications and representative stainings (mean $\pm$ SD, $n \geq 5$ mice/group, 2 independent experiments).

(B) EAE was induced in WT mice and N6-B cells were transferred at days –1, +10, or +17 p.i. Data show clinical scores (mean $\pm$ SEM, $n = 5$ mice/group for days –1, and $n \geq 10$ for day +10 p.i. and day +17 p.i., 2 independent experiments). At day +30 p.i., splenocytes were analyzed to quantify IL-17⁺CD4⁺ T cells and CD19⁺IL-10⁺ B cells, as in (A).

(C) EAE was induced in WT mice and N6-B cells from WT, *Il10*^{–/–}, or *H2*^{–/–} mice were transferred at day +17 p.i. Data show clinical scores (mean $\pm$ SEM, $n \geq 10$ mice/group, 2 independent experiments). At day +30 p.i., splenocytes were analyzed to quantify IL-17⁺CD4⁺ T cells and CD19⁺IL-10⁺ B cells, as in (A).

(D) CD25⁺ Treg cells were depleted at day –5. Data show representative FACS plots and quantification of CD25⁺FoxP3⁺ cells within CD4⁺ T cells and of FoxP3⁺ cells within CD4⁺ T cells at day 0 (mean $\pm$ SD, $n = 4$ mice/group from 2 independent experiments). Unpaired Mann-Whitney test comparing Ctrl group with a-CD25 group, * $p < 0.05$).

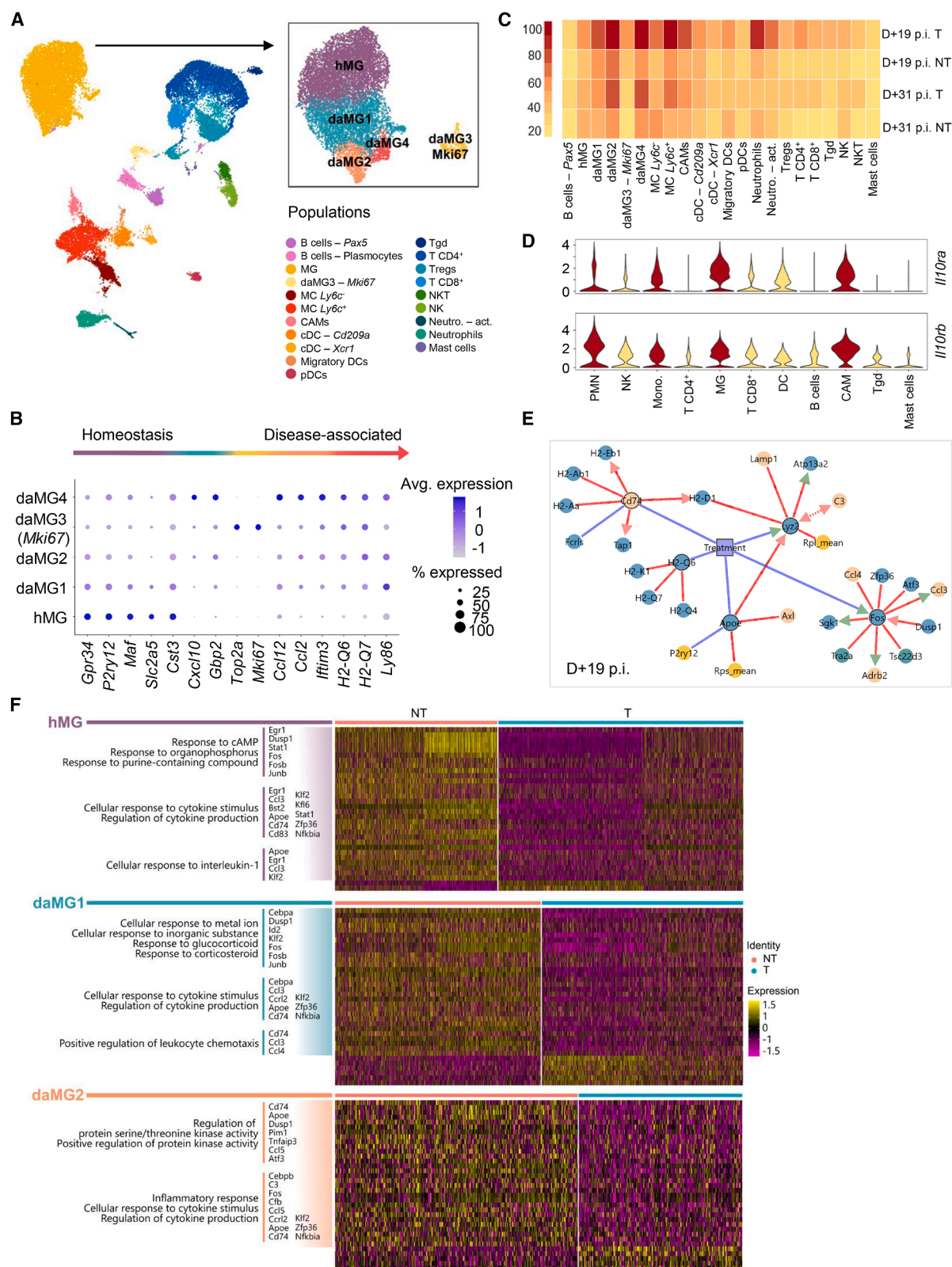
(E) EAE was induced in WT mice, including groups treated with a-CD25 (–Treg cells) at day –5, as in (D). N6-B cells were transferred at days –1, +10 p.i., or +17 p.i. Mice not depleted for Treg cells were used as control. Data show clinical score (mean $\pm$ SEM, $n \geq 10$ mice/group, 2 independent experiments).

(F) EAE was induced in Ly5.1[–]Ly5.2⁺ mice and iBreg cells from CD45.1⁺CD45.2[–] mice were transferred at day +17 p.i. Brains and spinal cords (SCs) were harvested at days +19 or +30 p.i. and CD45.1⁺CD45.2[–] iBreg cells quantified (mean $\pm$ SD, $n = 7$ mice/group from 2 independent experiments). A representative FACS plot of CD45.1/CD45.2 staining is shown for SC at day +19 p.i. of iBreg cells-treated mouse.

(G) EAE was induced in Ly5.1⁺Ly5.2[–] mice and iBreg cells from *Il10*.EGFP-*Il6*.tdTomato reporter mice (Ly5.1[–]Ly5.2⁺) were transferred at day +17 p.i. Organs were harvested at day +30 p.i. and *Il10*⁺ iBreg cells were quantified among viable/singlets/CD45⁺CD45.2⁺CD45.1[–] cells. Representative FACS plot for *Il10*/*Il6* expression in a SC sample is shown.

Statistical analyses were performed with Kruskal-Wallis test with Dunn's correction. * $p < 0.05$, *** $p < 0.001$, and **** $p < 0.0001$.

See also Figures S4 and S5 and Table S2.



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(MHC class II) (Figure 3C). Thus, N6-B cells drove disease resolution and suppressed CD4⁺ autoreactive T_H17 cells in an IL-10-dependent manner without requiring cognate interaction with CD4⁺ T cells.

Because IMIDs often involve Treg cell defects, we addressed whether N6-B cells could protect Treg cell-depleted mice. Mice treated with an anti-CD25 (Figure 3D) developed a worsening disease and were sacrificed around day +22 p.i. for ethical reasons (Figure 3E). The administration of N6-B cells at days –1 or +10 p.i. into Treg cells-depleted mice prevented severe disease development (Figure 3E). Remarkably, the injection of N6-B cells into Treg cells-depleted mice at day +17 p.i. restored remission from EAE (Figure 3E). N6-B cells can therefore compensate for a defect in Treg cells.

Based on the frequency of IL-10-expressing cells among N6-B cells, their stability, and their protective function in EAE, we named these cells induced regulatory B cells (iBreg cells).

The rapid effect of iBreg cells on ongoing EAE suggested that they acted in the CNS. Already 2 days after transfer near the peak of EAE, iBreg cells generated from CD45.1 congenic mice were found in spinal cord and brain of CD45.2 recipient mice, where they persisted for at least 2 weeks (Figure 3F). Transfer of iBreg cells generated from *Il10*.EGFP-*Il6*.tdTomato mice revealed that, 2 weeks after administration, CNS-infiltrating iBreg cells maintained a stable *Il10* expression while remaining *Il6* negative (Figure 3G). Further phenotyping showed that iBreg cells expressed high level of Ki-67 before injection (Figure S5A), yet were no longer Ki-67 positive in CNS at 2 weeks post-injection (Figure S5A). iBregs also displayed a B220^{lo}CD138^{hi} plasma cell phenotype in CNS at day 13 post-transfer (Figure S5B).

We next explored whether iBreg cells infiltration into the CNS required local inflammation. Upon administration into naive mice, iBreg cells accumulated in spleen but not in CNS (Figure S5C). When iBreg cells were injected at day –1 and mice were immunized at day 0, iBreg cells were also not found in CNS at day +7 p.i. (Figure S5D). However, the CNS displayed some inflammatory features at this early time point, notably the accumulation of neutrophils (Figure S5E), which was reduced in mice pre-treated with iBreg cells (Figure S5F). We conclude that iBreg cells require specific conditions to accumulate in the inflamed CNS, which are provided at the peak of the disease.

iBreg cells rapidly modulate the transcriptome of myeloid cells in the CNS of treated mice

We next asked how iBreg cells transferred at day +17 p.i. affected the transcriptomes of CNS CD45⁺ cells on days +19

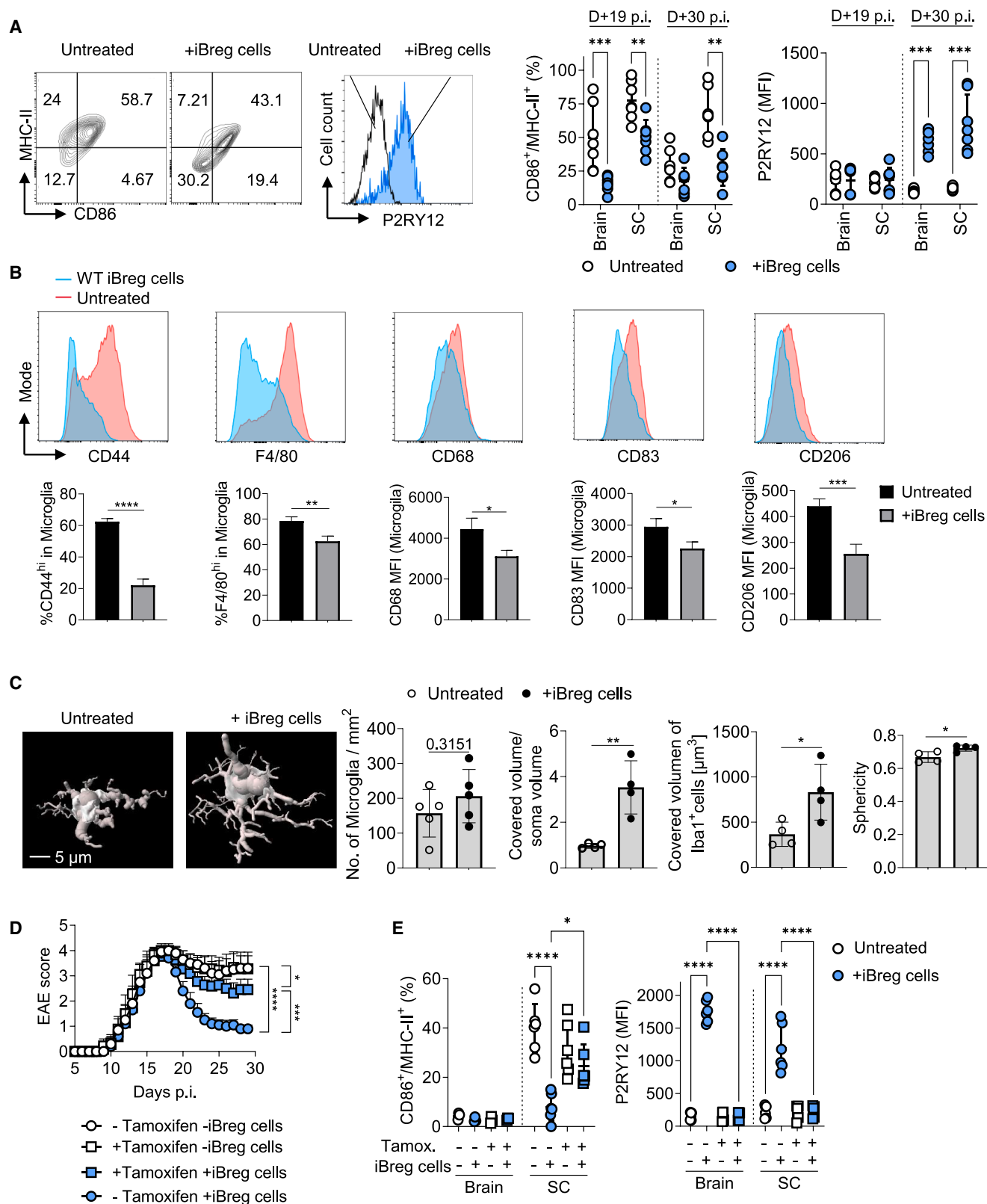
and +31 p.i. The treatment with iBreg cells was associated with a reduced clinical score (treated day +19 p.i.: 2 ± 0.4 ; non-treated day +19 p.i.: 3.6 ± 0.4 ; treated day +31 p.i.: 0.8 ± 0.3 ; non-treated day +31 p.i.: 2.6 ± 0.6), so that any transcriptional change could be due to a direct iBreg cell effect or indirectly due to the modulation of disease score. CD45⁺ cells from brain and spinal cord were analyzed by scRNA-seq (10 $\times$ Genomics). A UMAP distinguished microglia,^{24,25} monocytes, CNS-associated macrophages (CAMs), dendritic cells (DCs), T cells, B cells, natural killer (NK) cells, and neutrophils (Figure 4A; Table S3). Microglia transcripts were then re-processed in a separate UMAP and subjected to clustree analysis to deconvolute homeostatic and disease-associated microglia states, which were manually annotated using reference genes in brain (Figures 4A and 4B; Table S3) and spinal cord (data not shown).²⁶

B cells were found in CNS based on their expression of *Cd19* and iBreg cells were identified at days +19 and +31 p.i. with CD45.1 expression detected via cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq). iBreg cells were found in the plasmacyte cluster, where they accounted for 74% of cells in treated mice (Figure S6A). In comparison to endogenous B cells and plasmacytes, iBreg cells displayed a higher expression of genes from the N6 signature (*Il10*, *Net1*, *P2ry14*, *Phgdh*, and *Rnd3*) and plasmacyte-related genes (*Irf4*, *Jchain*, *Mzb1*, *Prdm1*, and *Xbp1*), as well as immunoglobulin genes (Figures S6B and S6C). iBreg cells were the major source of *Il10* derived from immune cells in the CNS of treated mice (Figure S6B).

CellPhoneDB was used to score potential interactions between iBreg cells and other cell types. This revealed microglia, CAMs, and monocytes as candidate targets of iBreg cells (Figure 4C). These cell types expressed the two IL-10R subunits (Figure 4D). Further analysis of the interactions between iBreg cells and microglia with NicheNet highlighted IL-10 as an important actor in this intercellular communication (Figure S6D). Microglia considered as a whole displayed remodeled transcriptomes in iBreg cells-treated mice compared with controls at 2 days post-treatment (day +19 p.i., Figure S6E). In contrast, there was little change in the transcriptome of CD4⁺ T cells between treated and untreated mice (data not shown). A machine learning algorithm called multivariate information-based inductive causation (MIIC) was applied to these data in order to infer causal networks and visualize the impact of the treatment on microglia.²⁷ Considering microglia as a whole, MIIC indicated an effect of iBreg cells on their antigen-presenting capacity (various

Figure 4. iBreg cells target microglia in the brain of EAE mice

- (A) EAE was induced in WT mice and iBreg cells from Ly5.1⁺Ly5.2[–] were transferred at day +17 p.i. CD45⁺ cells were FACS-isolated from brain at days +19 and +31 p.i. and analyzed by scRNA-seq. UMAP showing cell subsets identified among CD45⁺ cells (left) and UMAP showing microglia states after re-clustering all microglia (MG) (right). Individual UMAPs and numbers of cells per microglia states are shown in Table S3.
- (B) List of genes used to identify microglia states, as described in Jordão et al.,²⁶ combining brain samples obtained at days +19 and +31 p.i.
- (C) CellPhoneDB analysis of cell-cell interactions between the cluster “B cells-plasma cells,” which contains almost all iBreg cells in treated mice (see Figure S6A) and indicated cell types in treated (T) versus non-treated mice (NT). TScale shows the absolute number of interactions.
- (D) *Il10ra* (top) and *Il10rb* (bottom) expression levels in indicated subsets. Subsets with the highest number of interactions in (C) are highlighted in red.
- (E) MIIC causal networks inferred on the microglia population (excluding daMG-*Mki67*) at day +19 p.i. The highlighted “treatment” variable subnetwork shows inferred causal and correlated effects of the iBreg cells treatment on microglia genes expression.
- (F) Heatmaps and gene set enrichment analysis with associated genes based on differentially expressed genes in indicated microglia states between T and NT mice analyzed at day +19 p.i. Heatmaps with all annotated genes are provided in Table S3.
- See also Figure S6 and Table S3.



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MHC class I and MHC class II molecules, *Cd74*, and *Tap1*), lipid metabolism (*Apoe*, *Apoc1*, *Apoc2*, and *Apoc4*), lysosome M (*Lyz2*), complement (*C3*), chemokines (*Ccl3* and *Ccl4*), and Fos-controlled genes (Figure 4E). A gene set enrichment analysis on different microglial clusters indicated that iBreg cells also regulated pathways related to cytokine and inflammation as well as aging and lipid metabolism (Figure 4F; Table S3). 26 DEGs were shared between homeostatic microglia (hMG), disease-associated microglia state 1 (daMG1), and daMG2 states, including *Cd74*, *Dusp1*, *Fosb*, *Fcrls*, *H2-Ab1*, *Il1b*, *Klf4*, *H2-Eb1*, *Zfp36*, *AW112010*, *Klf2*, *Junb*, *Ier2*, *2410006H16Rik*, *Lgals3bp*, *Ccl2*, *Tnfrsf3*, *H2-Aa*, *Fos*, *Rpl22l1*, *Pim1*, *Ccl5*, *Atf3*, *Lyz2*, *Apoe*, and *Neat1* (Table S3). DEGs with a lower expression in cells from the treated group included genes coding for molecules involved in antigen presentation (*Cd74*, *H2-Ab1*, *H2-Eb1*, and *H2-Aa*), immediate-early activation genes (*Fos*, *Fosb*, *Junb*, *Ier2*, and *Atf3*), and genes coding for inflammatory mediators (*Il1b* and *Ccl5*). The only gene increased in all three states upon treatment was *Fcrls*, which is expressed at high level in microglia,²⁸ possibly reflecting an improvement of microglia health.²⁹ *Fcrls* was also increased in daMG4. Noteworthy, iBreg cells modulated in microglia the expression of 21 of the 24 genes identifying the shared “disease-inflammatory macrophages signature” defined by Silvén et al.,³⁰ which was found in myeloid cells (identified initially as macrophages or microglia) in different studies focused on ageing and neurodegenerative disease (Alzheimer’s disease)^{31–33} (Table S3). This suggests a therapeutic potential for iBreg cells beyond EAE.

The control of long-lived CNS-resident myeloid cells (microglia and CAMs) is required for iBreg cells to reverse EAE in recipient mice

The analysis of CNS myeloid cells by flow cytometry confirmed that iBreg cells acted on microglia ($CD45^{mid}CD11b^{mid}Ly-6C^{neg}CX3CR1^{hi}$), reducing the frequency of cells co-expressing CD86 and MHC class II (Figures 5A and S7A). The expression of CD86 on microglia was confirmed by comparison to neutrophils (Figure S7B). This treatment also restored the expression of P2RY12, a marker of resting microglia whose expression goes down in parallel to their activation³⁴ (Figure 5A), without affecting microglia cell number (Figure S7A). $CD45^{hi}CD11b^{hi}Ly-6C^{neg}CX3CR1^{mid}$ CAMs and $CD45^{hi}CD11b^{hi}Ly-6C^{hi}CX3CR1^{mid}$ mono-

cytes also showed a reduced CD86/MHC class II expression in iBreg cells-treated mice (Figure S7C). To characterize further the effect of iBreg cells on CNS myeloid cells, we applied a spectral flow cytometry panel measuring 20 markers (Table S4) at 2 days post-treatment with iBregs (day +19 p.i.). The analysis of individual activation markers showed a reduction of the frequency of $CD44^{hi}$ cells and $F4/80^{+}$ microglia as well as a global reduction of CD68, CD83, and CD206 expression in microglia from iBreg cells-treated mice, compared with microglia from control mice, suggesting a reduction of their activation status (Figure 5B). The unsupervised analysis of these data identified 8 clusters corresponding to microglia (clusters 5 and 6) and monocytes/macrophages (the other clusters) and confirmed the modulation of microglia as well as monocytes by the treatment. Focusing on the most abundant clusters (representing >10% of total cells), we found that the treatment with iBreg cells induced an increase and a reduction of the relative abundances of microglia cluster 6 and of monocyte cluster 7 within live $CD11b^{+}Ly6G^{-}$ cells, respectively (Figure S7D). Compared with all other microglia, cells in cluster 6 displayed an increased expression of P2RY12 and CX3CR1 and a lower expression of CD11a, in agreement with a return toward a homeostatic state (Figure S7D). This was corroborated by changes in microglia morphology at day +30 p.i., with microglia from iBreg cells-treated mice displaying an increased covered volume over soma volume, covering an increased volume, and showing a slightly higher sphericity (Figure 5C).

Monocytes from cluster 7 displayed a higher expression of CD11c compared with other monocytes (Figure S7D), suggesting that these cells, which were less abundant in treated mice, were engaged in DC differentiation. Thus, treatment with iBreg cells might reduce the accumulation of monocyte-derived DC in the inflamed CNS. These data support the notion that iBreg cells modulate CNS myeloid cells, including monocytes and microglia.

The role of monocytes in neuroinflammation has been previously documented,^{35–37} while the role of microglia in the transition from acute to chronic disease remains less defined.³⁸ We thus focused on microglia for further analysis. To delineate the role of IL-10R signaling in microglia for the iBreg cells effect, *Cx3cr1^{creER};Il10ra^{fl/fl}* mice were treated with tamoxifen to mutate the *Il10ra* gene and used 6–8 weeks later to restrict *Il10ra* deficiency to long-lived cells.³⁹ Mice with the selective deletion of

Figure 5. IL-10R signaling in microglia is required for the iBreg cells therapeutic effect

(A) EAE was induced in WT mice and iBreg cells from $Ly5.1^{+}Ly5.2^{-}$ mice were transferred at day +17 p.i. Brain and spinal cord (SC) were harvested at days +19 or +30 p.i., and viable/singlets/ $CD45^{mid}CD11b^{mid}Ly-6C^{neg}CX3CR1^{hi}$ microglia were stained for CD86/MHC class II and P2RY12 in treated (+ iBreg cells) and untreated mice. Data show representative FACS plots and quantifications (mean $\pm$ SD, $n = 7$ mice/group, 2 independent experiments).
(B) EAE was induced in WT mice and iBreg cells were transferred at day +17 p.i. Mice were analyzed at day +19 p.i. Data show representative FACS histograms (top) and quantifications for activation markers in SC microglia (bottom) (mean $\pm$ SEM, $n \geq 10$ mice/group from 2 independent experiments). Mann-Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.
(C) EAE was induced in WT mice and iBreg cells were transferred at day +17 p.i. Mice were analyzed at day +30 p.i. Data show representative IMARIS image reconstruction of microglia and quantification of microglia density as well as morphological parameters ($n = 4$ per group, up to 5 microglia per animal reconstructed) presented as mean $\pm$ SEM. Unpaired t test. * $p < 0.05$ and ** $p < 0.01$.
(D) EAE was induced in *Cx3cr1^{creER};Il10ra^{fl/fl}* mice pre-treated or not with tamoxifen (Tamox.), and iBreg cells were transferred at day +17 p.i. Data show clinical scores (mean $\pm$ SEM, $n \geq 9$ mice/group, 2 independent experiments).
(E) Viable/singlets/ $CD45^{mid}CD11b^{mid}Ly-6C^{neg}CX3CR1^{hi}$ microglia from mice treated as indicated were stained for CD86/MHC class II and P2RY12 at day +30 p.i. (mean $\pm$ SD, $n = 6$ mice/group, 2 independent experiments).
Statistical analyses were with a two-way ANOVA followed by Tukey’s multiple comparisons test (A and E) or with a Kruskal-Wallis test with Dunn’s correction (D). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.
See also Figure S7 and Table S4.

Il10ra in long-lived microglia and CAMs had a minor response to iBreg cells treatment (Figure 5D). This was associated with the maintenance of activated microglia and CAM phenotypes indicated by the elevated expression of CD86/MHC class II as well as a low expression of P2RY12 (Figures 5E and S7E). However, mice with an *Il10ra* deficiency in microglia and treated with iBreg cells displayed a reduced expression of CD86/MHC class II in monocytes (Figure S7E). Thus, the control of short-lived monocytes is insufficient to reverse disease as long as long-lived microglia (and CAMs) are unreached by iBreg cells-derived IL-10.

iBreg cells thus provide an IL-10 delivery platform to control the deleterious function of CNS-resident myeloid cells that drive chronic CNS autoimmunity.

iBreg cells drive disease resolution in a B cell-dependent model of CNS autoimmunity

Because B cells can be deleterious in MS,⁴⁰ we next evaluated the function of iBreg cells in a B cell-dependent EAE model using recombinant human MOG_{1–125} (hMOG_{1–125}) to induce disease.⁴¹ The administration of iBreg cells also triggered disease resolution in this model (Figure 6A), which was associated with iBreg cells accumulating in CNS as B220^{lo}CD138^{hi}Ki-67^{neg} plasma cells (Figures S8A and S8B). Disease improvement was linked to the downregulation of CD86/MHC class II in microglia, CAMs, and monocytes and to the increased expression of P2RY12 in microglia (Figures 6B, S8C, and S8D). iBreg cells had no effect on anti-hMOG_{1–125} antibodies levels (Figure S8E).

We next addressed whether iBreg cells produced from a B cell compartment containing autoreactive B cells were protective. To this end, we generated iBreg cells from the spleen of mice immunized with hMOG_{1–125}. Such iBreg cells were as efficient as iBreg cells produced from naive mice against EAE (Figure 6C), controlling similarly the activation of microglia (Figure 6D), CAMs, and monocytes (Figures S8F and S8G), without leading to increased anti-hMOG_{1–125} antibodies in treated mice (Figure S8H).

We infer from these results that iBreg cells keep their therapeutic benefit in CNS autoimmune disease involving pathogenic autoreactive B cells.

These results led us to consider that iBreg cells might operate in an antigen-independent manner. We thus tested whether monoclonal iBreg cells expressing an irrelevant BCR were also protective. To this end, iBreg cells were produced from MD4xRAG^{−/−} mice expressing a single transgenic BCR specific for hen egg lysozyme. These iBreg cells displayed a comparable therapeutic effect as polyclonal iBreg cells from WT mice and achieved a similar control of myeloid cell activation in the CNS (Figures 6E, 6F, S8I, and S8J).

We conclude that BCR (auto)reactivity does not play a key role in iBreg cells effect, in contrast to what is known about Treg cells.

Identification of analog small molecules inducing IL-10 expression in human B cells

To prepare for the clinical translation of these findings, we tested the capacity of C3, N6, and 13 additional chemical analogs to increase IL-10 expression in human B cells. This led to the identification of N4 as inducer of IL-10 expression, which achieved a superior effect than C3 and N6 in human B cells (Figure S9A). To obtain additional active compounds, new structural analogs related to N4 were synthesized (see STAR Methods). We de-

signed 3 series of compounds related to N4. We prepared 130 compounds based on a N-methylbenzimidazole scaffold (series 1), 90 compounds based on an imidazopyridine scaffold (series 2), and 23 compounds based on a N-methyldeazapurine scaffold (series 3) and identified 7 additional compounds inducing IL-10 (Figure S9B). The analysis of human B cells activated with compound 1n showed that the increased IL-10 expression could also be detected by flow cytometry (Figures S9C and S9D). Noteworthy, 1n also augmented IL-10 expression in B cells from healthy donors (HDs), patients with clinically isolated syndrome (CIS), or relapsing remitting multiple sclerosis (RRMS) (Table 1; Figures 7A and 7B), erasing the defect in IL-10 production seen in B cells from these patients compared with controls (Figure 7C).

We conclude that identified compounds are active in human B cells and might be useful to restore B cell-mediated immune regulation in patients suffering from defects in this protective axis.

DISCUSSION

IL-10-producing B cells have protective functions in multiple models of IMIDs and are defective in subgroups of patients with such disorders.⁶ It is thus of interest to develop a cell therapy based on regulatory B cells. This would benefit from having a strategy to produce a cell product highly enriched in stable IL-10-producing cells. Here, we identified IL-10-inducing compounds and a strategy to easily generate such B cells in mice, which we term iBreg cells. iBreg cell administration to recipient mice near the peak of EAE led to rapid disease resolution.

IL-10-producing B cells display a plasma cell phenotype in many diseases. In EAE B cells providing protective IL-10 show a plasmacyte phenotype in lymph nodes,⁴² spleen,²¹ and CNS.⁴³ Plasmacytes are also a major source of B cell-derived IL-10 in other autoimmune, infectious, and malignant disease models.^{4,13,44–46} In MS patients, IL-10-producing plasmacytes are found in active CNS lesions.^{47,48} iBreg cells progressively acquired a plasmacyte phenotype in treated mice, suggesting that our approach might generate cells mimicking the IL-10-expressing plasmacytes naturally occurring in diseases in mice⁴³ and humans.^{47,48}

The differentiation of B cells into *Il10*-expressing iBreg cells *in vitro* was, however, not strictly dependent on their plasmacyte differentiation. These observations are consistent with the results from Matsumoto et al. on the role of *Prdm1* and *Irf4* for IL-10 expression in B cells activated through TLR4 and BCR for 72 h.⁴²

The protective function of iBreg cells required IL-10 but was independent of MHC class II and of their antigen-reactivity. This may relate to the fact that iBreg cells were already well-advanced *in vitro* in their differentiation trajectory toward IL-10-producing plasmacytes and did not depend on T cell-derived survival or differentiation signals *in vivo*.

iBreg cells modulated microglia, which have complex roles⁴⁹ and can be detrimental in EAE.^{15,50–53} iBreg cells modulated molecules important for microglia pathogenic functions during EAE and neurodegenerative diseases, such as the nuclear factor κ B (NF- κ B) and the triggering receptor expression on myeloid cells 2 (TREM2)/apolipoprotein E (ApoE) pathways.^{54,55} In addition, iBreg cells modulated the expression of genes identified as

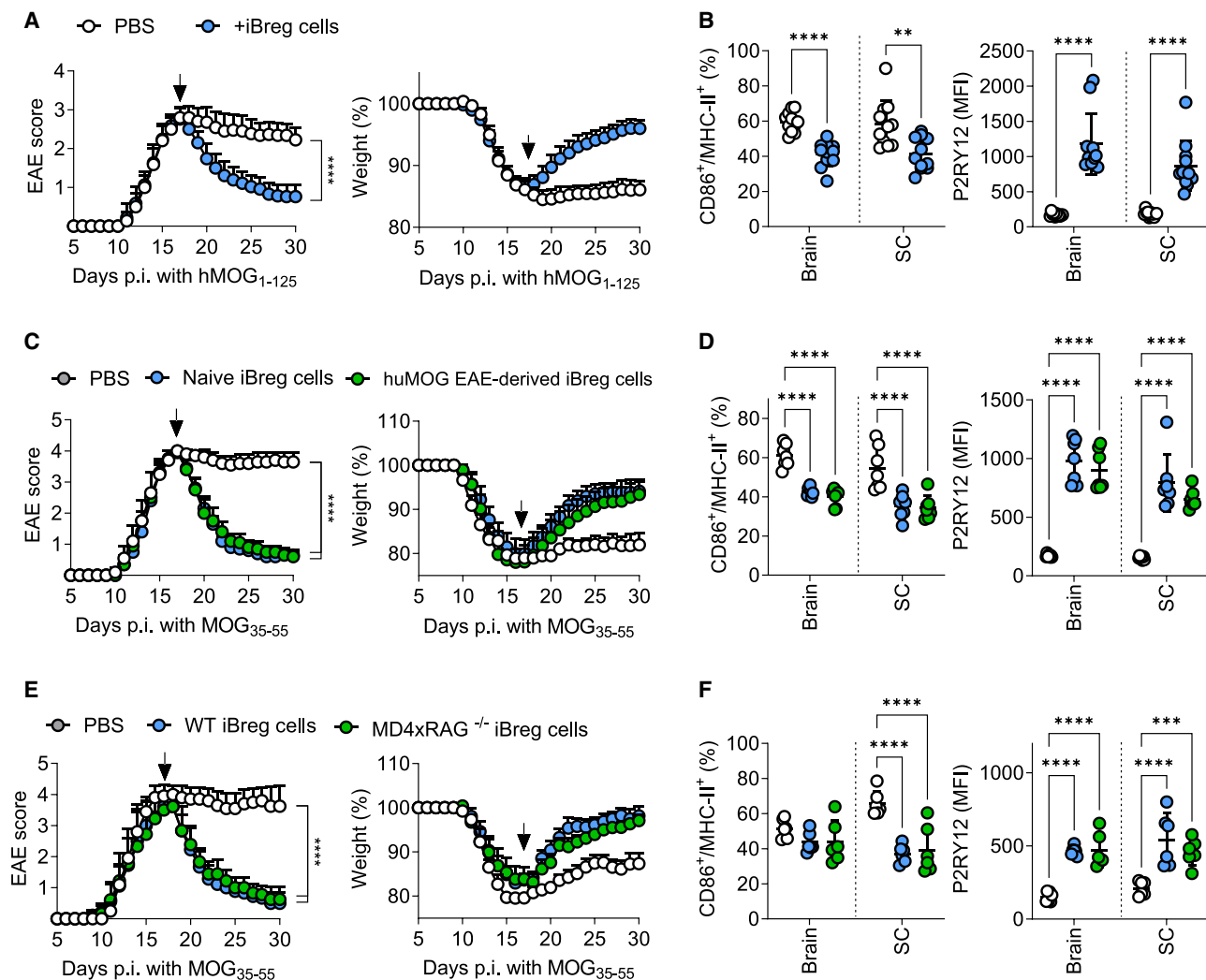


Figure 6. BCR (auto)reactivity does not play a key role in iBreg cells effect

(A) EAE was induced in WT mice with hMOG₁₋₁₂₅, and iBreg cells from Ly5.1⁺Ly5.2⁻ mice were transferred at day +17 p.i. Clinical score and weight loss are presented (mean ± SEM, *n* ≥ 22 mice/group, 4 independent experiments).

(B, D, and F) Viable/singlets/CD45^{mid}CD11b^{mid}Ly6C^{neg}CX3CR1^{hi} microglia were quantified for indicated markers in indicated organs at day +30 p.i. (mean ± SD). (B) *n* = 11 mice/group, 4 independent experiments for mice in (A), (D) *n* = 7 mice/group, 2 independent experiments for mice in (C), and (F) *n* = 6 mice/group, 2 independent experiments for mice in (E).

(C) EAE was induced in WT mice with MOG₃₅₋₅₅, and iBreg cells from naive mice or from mice that previously experienced EAE induced with hMOG₁₋₁₂₅ (B cells isolated at day +30 p.i.) were transferred at day +17 p.i. Clinical score and weight loss are presented (mean ± SEM, *n* = 10 mice/group, 2 independent experiments).

(E) EAE was induced in WT mice with MOG₃₅₋₅₅, and iBreg cells from WT or MD4xRAG^{-/-} mice were transferred at day +17 p.i. Clinical score and weight loss are presented (mean ± SEM, *n* ≥ 9 mice/group, 2 independent experiments).

Statistical analyses were performed with an unpaired Mann-Whitney test (A and B) or with a Kruskal-Wallis test with Dunn's correction (C-F). ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001.

See also Figure S8.

the signature of DIMs that accumulated in brain during ageing and neurodegenerative disease (Alzheimer's disease).³⁰ iBreg cells might thus be able to erase the deleterious functions of microglia and CNS macrophages during ageing and neurodegenerative diseases such as Alzheimer's disease.

IL-10 is a key mediator of the protective function of iBreg cells, and microglia were identified as an important target. IL-10 has been shown to protect mice from several CNS disorders by acting on microglia. IL-10 prevented microglia hyperactivation

after endotoxin challenge³⁸ and microglia-specific gene delivery of IL-10 inhibited neuroinflammation and neurodegeneration in a mouse model of Parkinson's disease.⁵⁶ It was recently shown that a mutated version of IL-10 could improve age-related cognitive loss in mice.⁵⁷ It is noteworthy that the utilization of IL-10 on its own in EAE has, however, yielded conflicting results with regards to its capacity to reverse disease.^{58,59} Several explanations could account for this including differences in dose, localization, and duration of IL-10 provision. It is also possible that

Table 1. Characteristics of human biological samples

ID	Gender	Treatment 1	Treatment 2	Treatment 3	Wash out (last treatment and sampling, days)	Age (disease onset, years)	Age (sampling, years)
HD n1	M	–	–	–	–	N/A	35
HD n2	F	–	–	–	–	N/A	50
HD n3	F	–	–	–	–	N/A	30
HD n4	F	–	–	–	–	N/A	18
HD n5	F	–	–	–	–	N/A	35
HD n6	F	–	–	–	–	N/A	32
HD n7	F	–	–	–	–	N/A	33
HD n8	F	–	–	–	–	N/A	39
HD n9	F	–	–	–	–	N/A	24
HD n10	F	–	–	–	–	N/A	48
HD n11	F	–	–	–	–	N/A	35
HD n12	F	–	–	–	–	N/A	54
HD n13	F	–	–	–	–	N/A	26
HD n14	F	–	–	–	–	N/A	28
HD n15	M	–	–	–	–	N/A	45
CIS n1	F	none	–	–	–	53	53
CIS n2	F	none	–	–	–	30	30
CIS n3	F	none	–	–	–	18	18
CIS n4	F	none	–	–	–	32	32
CIS n5	F	none	–	–	–	33	33
CIS n6	F	none	–	–	–	39	39
CIS n7	F	none	–	–	–	24	24
CIS n8	F	none	–	–	–	48	48
CIS n9	F	none	–	–	–	35	35
CIS n10	F	none	–	–	–	62	62
CIS n11	F	none	–	–	–	25	25
CIS n12	F	none	–	–	–	28	28
CIS n13	M	none	–	–	–	45	45
RRMSn1	M	Copaxone	Betaferon	–	883	25	31
RRMSn2	F	Copaxone	–	–	78	22	21
RRMSn3	F	Tecfidera	–	–	209	34	35
RRMSn4	F	Rebif	Avonex	–	570	22	31
RRMSn5	F	Betaferon	–	–	1,152	38	43
RRMSn6	F	IFN- β	–	–	78	17	27
RRMSn7	F	Avonex	Rebif	–	395	22	43
RRMSn8	F	Betaferon	Copaxone	–	355	34	53
RRMSn9	F	Betaferon	–	–	77	21	27
RRMSn10	F	Copaxone	Rebif	Copaxone	70	28	33
RRMSn11	M	Copaxone	–	–	262	24	42
RRMSn12	F	none	–	–	–	33	33
RRMSn13	M	none	–	–	–	42	42
RRMSn14	F	none	–	–	–	23	23
RRMSn15	F	none	–	–	–	22	24
RRMSn16	F	none	–	–	–	40	40
RRMSn17	F	methylprednisolone	–	–	92	22	22
RRMSn18	M	methylprednisolone	–	–	31	42	42
RRMSn19	M	none	–	–	–	30	30

(Continued on next page)

Table 1. Continued

ID	Gender	Treatment 1	Treatment 2	Treatment 3	Wash out (last treatment and sampling, days)	Age (disease onset, years)	Age (sampling, years)
RRMSn20	F	none	–	–	–	43	43
RRMSn21	F	none	–	–	–	55	55

Characteristics of human biological samples, related to Figure 7. M, male; F, female; N/A, not applicable; HD, healthy donor; CIS, clinically isolated syndrome; and RRMS, relapsing remitting multiple sclerosis.

iBreg cells produce other factors that synergize with IL-10 to control established disease.

It is important that iBreg cells also modulated CAMs and monocytes. Monocytes are known to play pathogenic roles during EAE, and their depletion using anti-C-C chemokine receptor type 2 (CCR2) (which led to the depletion of

CCR2⁺CD11b⁺Ly6C^{hi} monocytes) starting at the peak of the disease led to improvement of clinical symptoms.³⁷ Testing the relevance of IL-10R signaling in these cells is, however, complicated because mice lacking *Il10ra* in monocytes spontaneously develop colitis at a young age,^{60,61} which would confound the interpretation of the results for EAE.

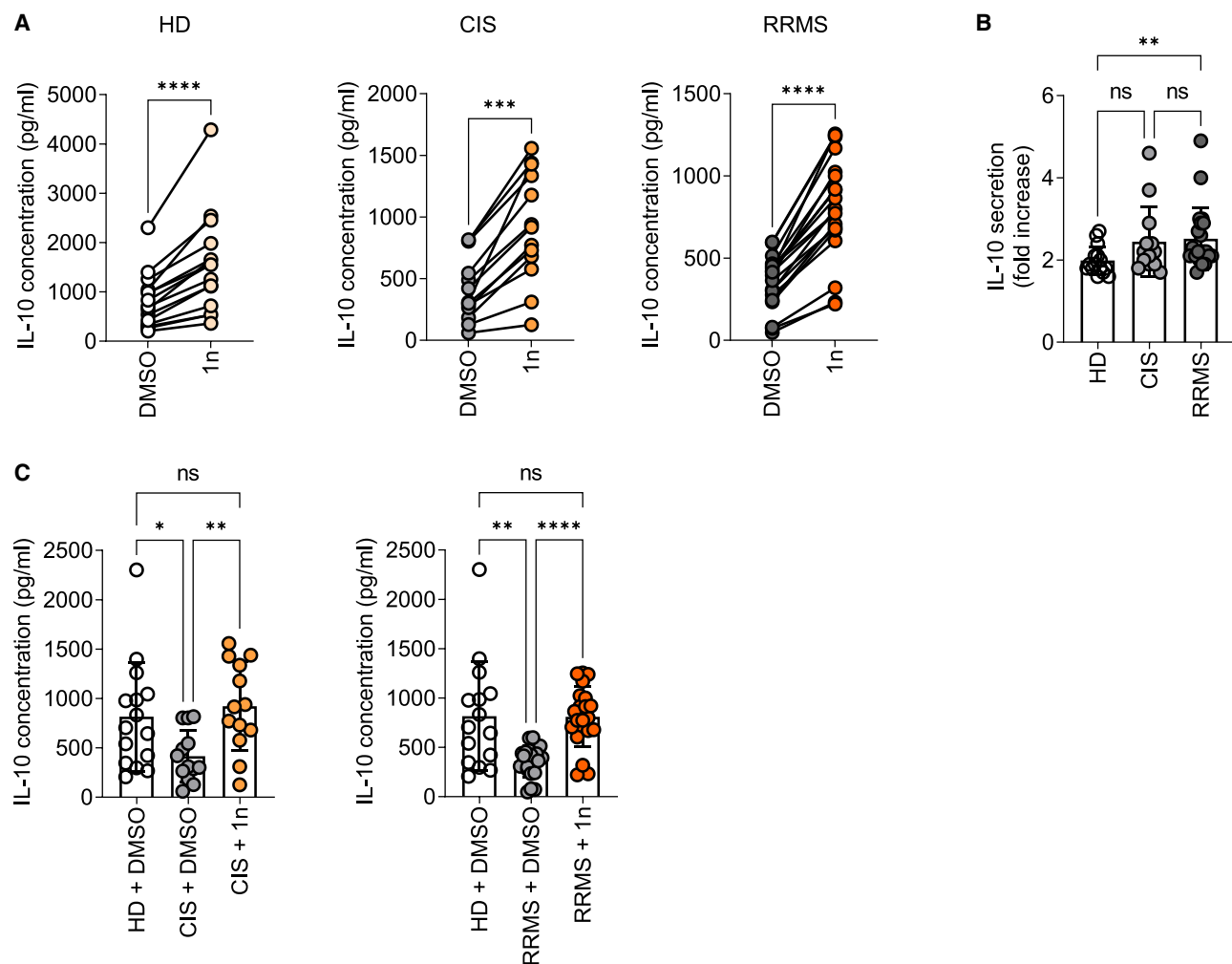


Figure 7. Compound 1n corrects IL-10 secretion defects in B cells from CIS and MS donors

(A) B cells were isolated from PBMCs of healthy donors (HDs), clinically isolated syndrome (CIS), or relapsing remitting multiple sclerosis (RRMS) patients and stimulated with CpG/anti-IgM for 3 days. Medium was supplemented with 1n (0.25 μ M) or DMSO. Supernatants were analyzed by ELISA for IL-10.

(B) IL-10 fold-increase (relative to DMSO-control condition) for indicated groups (mean $\pm$ SD).

(C) IL-10 concentration in supernatants for B cells from indicated groups is shown (mean $\pm$ SD).

HD $n = 15$, CIS $n = 13$, and RRMS $n = 21$. Paired samples were analyzed with a Wilcoxon test (A); otherwise, statistical analyses were performed with a Kruskal-Wallis test with Dunn's correction (B and C). ns > 0.05, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

See also Figure S9.

Limitations of the study

A first limitation is the lack of knowledge of the target of the compounds. A second limitation is the fact that we have not established a protocol to produce highly enriched and stable IL-10-expressing human B cells. Third, deciphering the molecular and cellular networks within the CNS that are mobilized by iBreg cells to reverse disease might offer novel insights into the pathogenesis of these diseases and highlight novel therapeutic possibilities. We have focused here on microglia, but our data also show that monocytes and CAMs are modulated by the iBreg cells treatment. Fourth, the precise identity of the long-lived CX3CR1-expressing myeloid cells targeted by iBreg cells remain to be defined. Finally, the administration of IL-10 into the CNS at the peak of EAE has yielded conflicting results. It remains to be established whether iBreg cells rely on other mechanisms than IL-10 to reverse disease.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Simon Fillatreau (simon.fillatreau@inserm.fr).

Materials availability

Certain materials are shared with academic and non-profit research organizations for research and educational purposes only under an MTA to be discussed in good faith with the recipient.

Data and code availability

- scRNA-seq data, Affymetrix gene array data, and ATAC-seq data have been deposited at GEO and are publicly available as of the date of publication. Accession numbers are listed in the [key resources table](#).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.
- Affymetrix gene array data for B cells treated with DMSO or N6 have been deposited at NCBI GEO depository and are accessible with the accession number GEO: GSE220719.
- scRNA-seq data for CNS CD45⁺ cells (brain and spinal cord) have been deposited at NCBI GEO depository and are accessible with the accession number GEO: GSE222061.
- scRNA-seq data for B cells activated *in vitro* have been deposited at NCBI GEO depository and are accessible with the accession number GEO: GSE219282.
- ATAC-seq data for *in vitro* stimulated B cells have been deposited at NCBI GEO depository and are accessible with the accession number GEO: GSE222227.

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AUTHOR CONTRIBUTIONS

B.M. performed experiments for the establishment of the protocol to generate iBreg cells and their assessment in cell therapy. V.D.D. realized the screen and experiments leading to C3, N6, and N4, as well as short-term cultures of B cells. B.T.C. performed EAE experiments to address the effect of iBreg cells on CNS cells. J.P.v.K. and E.S. provided the chemical library and helped with the identification of C3 analogs. Z.W. helped with EAE experiments. D.A.-S. performed the unsupervised flow cytometry analyses. A.H. and S.F. established the high-throughput screening platform. A.J. and J.W. performed the ATAC-seq experiments and analyzed corresponding data. S.B., C.B., and F.M.-B. synthesized novel IL-10-inducing compounds for human B cells. L.D. and H.I. analyzed the scRNA-seq data from CD45⁺ CNS cells. E.G., K.B., and M.P. performed the histology and the analysis of CNS samples. L.S.A. provided support for the spectral analysis of the CNS myeloid cell compartment. G.C. analyzed the scRNA-seq data on *in vitro* activated B cells. Y.T. contributed to the characterization of N6 on B cells activated *in vitro*. N.T.N. and L.S.W. characterized the effect of novel compounds on human B cells. M.E.B., M.L., and M.M. contributed to scRNA-seq analyses. L.J. and P.B. analyzed Affymetrix data. S.S., R.G.G., and T.H.W. contributed to BCR repertoire analyses. R.L. and R.M. contributed to analyses of B cells from CIS and RRMS patients as well as controls. S.J. contributed to analysis of the expression of IL-10R on CNS myeloid cells during EAE. S.F. designed the study and supervised the research. B.M. and S.F. wrote the manuscript. All authors contributed to the editing and correction of the manuscript.

DECLARATION OF INTERESTS

S.F., B.M., V.D.D., F.M.-B., C.B., and S.B. are authors on patents describing compounds to modulate protein expression in immune cells (EP22169529, EP22169542, and EP22169555) and a patent application (EP26305238.3) related to the work described here.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

- Visperas, A., and Vignali, D.A.A. (2016). Are Regulatory T Cells Defective in Type 1 Diabetes and Can We Fix Them? *J. Immunol.* 197, 3762–3770. <https://doi.org/10.4049/jimmunol.1601118>.
- Grayling, M.J., Bigirimurame, T., Cherlin, S., Ouma, L., Zheng, H., and Wason, J.M.S. (2021). Innovative trial approaches in immune-mediated inflammatory diseases: current use and future potential. *BMC Rheumatol.* 5, 21. <https://doi.org/10.1186/s41927-021-00192-5>.
- Fillatreau, S., Sweeney, C.H., McGeachy, M.J., Gray, D., and Anderton, S.M. (2002). B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* 3, 944–950. <https://doi.org/10.1038/ni833>.
- Neves, P., Lampropoulou, V., Calderon-Gomez, E., Roch, T., Stervbo, U., Shen, P., Kühl, A.A., Lodenkemper, C., Haury, M., Nedospasov, S.A., et al. (2010). Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during *Salmonella typhimurium* infection. *Immunity* 33, 777–790. <https://doi.org/10.1016/j.immuni.2010.10.016>.
- Yanaba, K., Bouaziz, J.D., Haas, K.M., Poe, J.C., Fujimoto, M., and Tedder, T.F. (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 28, 639–650. <https://doi.org/10.1016/j.immuni.2008.03.017>.
- Mauri, C., and Bosma, A. (2012). Immune regulatory function of B cells. *Annu. Rev. Immunol.* 30, 221–241. <https://doi.org/10.1146/annurev-immunol-020711-074934>.
- Mauri, C., and Menon, M. (2017). Human regulatory B cells in health and disease: therapeutic potential. *J. Clin. Invest.* 127, 772–779. <https://doi.org/10.1172/JCI85113>.
- Lampropoulou, V., Hoehlig, K., Roch, T., Neves, P., Calderón Gómez, E., Sweeney, C.H., Hao, Y., Freitas, A.A., Steinhoff, U., Anderton, S.M., et al. (2008). TLR-activated B cells suppress T cell-mediated autoimmunity. *J. Immunol.* 180, 4763–4773. <https://doi.org/10.4049/jimmunol.180.7.4763>.
- Duddy, M., Niino, M., Adatia, F., Hebert, S., Freedman, M., Atkins, H., Kim, H.J., and Bar-Or, A. (2007). Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J. Immunol.* 178, 6092–6099. <https://doi.org/10.4049/jimmunol.178.10.6092>.
- Correale, J., Farez, M., and Razzitte, G. (2008). Helminth infections associated with multiple sclerosis induce regulatory B cells. *Ann. Neurol.* 64, 187–199. <https://doi.org/10.1002/ana.21438>.
- Mauri, C., Gray, D., Mushtaq, N., and Londei, M. (2003). Prevention of arthritis by interleukin 10-producing B cells. *J. Exp. Med.* 197, 489–501. <https://doi.org/10.1084/jem.20021293>.
- Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R.S., and Bhan, A.K. (2002). Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 16, 219–230. [https://doi.org/10.1016/s1074-7613\(02\)00274-1](https://doi.org/10.1016/s1074-7613(02)00274-1).
- Scapini, P., Lamagna, C., Hu, Y., Lee, K., Tang, Q., DeFranco, A.L., and Lowell, C.A. (2011). B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice. *Proc. Natl. Acad. Sci. USA* 108, E823–E832. <https://doi.org/10.1073/pnas.1107913108>.
- Hoehlig, K., Shen, P., Lampropoulou, V., Roch, T., Malissen, B., O'Connor, R., Ries, S., Hilgenberg, E., Anderton, S.M., and Fillatreau, S. (2012). Activation of CD4⁺ Foxp3⁺ regulatory T cells proceeds normally in the absence of B cells during EAE. *Eur. J. Immunol.* 42, 1164–1173. <https://doi.org/10.1002/eji.201142242>.
- Nissen, J.C., Thompson, K.K., West, B.L., and Tsirka, S.E. (2018). Csf1R inhibition attenuates experimental autoimmune encephalomyelitis and promotes recovery. *Exp. Neurol.* 307, 24–36. <https://doi.org/10.1016/j.expneurol.2018.05.021>.
- Dagkonaki, A., Avloniti, M., Evangelidou, M., Papazian, I., Kanistras, I., Tseveleki, V., Lampros, F., Tselios, T., Jensen, L.T., Möbius, W., et al. (2020). Mannan-MOG35–55 Reverses Experimental Autoimmune Encephalomyelitis, Inducing a Peripheral Type 2 Myeloid Response, Reducing CNS Inflammation, and Preserving Axons in Spinal Cord Lesions. *Front. Immunol.* 11, 575451. <https://doi.org/10.3389/fimmu.2020.575451>.
- Mader, M.M.D., Napole, A., Wu, D., Atkins, M., Scavetti, A., Shibuya, Y., Foltz, A., Hahn, O., Yoo, Y., Danziger, R., et al. (2024). Myeloid cell replacement is neuroprotective in chronic experimental autoimmune encephalomyelitis. *Nat. Neurosci.* 27, 901–912. <https://doi.org/10.1038/s41593-024-01609-3>.
- Vollmer, J., Weeratna, R., Payette, P., Jurk, M., Schetter, C., Laucht, M., Wader, T., Tluk, S., Liu, M., Davis, H.L., et al. (2004). Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. *Eur. J. Immunol.* 34, 251–262. <https://doi.org/10.1002/eji.200324032>.
- Barr, T.A., Shen, P., Brown, S., Lampropoulou, V., Roch, T., Lawrie, S., Fan, B., O'Connor, R.A., Anderton, S.M., Bar-Or, A., et al. (2012). B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J. Exp. Med.* 209, 1001–1010. <https://doi.org/10.1084/jem.20111675>.
- Arkatkar, T., Du, S.W., Jacobs, H.M., Dam, E.M., Hou, B., Buckner, J.H., Rawlings, D.J., and Jackson, S.W. (2017). B cell-derived IL-6 initiates spontaneous germinal center formation during systemic autoimmunity. *J. Exp. Med.* 214, 3207–3217. <https://doi.org/10.1084/jem.20170580>.
- Shen, P., Roch, T., Lampropoulou, V., O'Connor, R.A., Stervbo, U., Hilgenberg, E., Ries, S., Dang, V.D., Jaimes, Y., Daridon, C., et al. (2014). IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 507, 366–370. <https://doi.org/10.1038/nature12979>.
- Ricci, D., Gidalevitz, T., and Argon, Y. (2021). The special unfolded protein response in plasma cells. *Immunol. Rev.* 303, 35–51. <https://doi.org/10.1111/imr.13012>.
- Benkert, P., Meier, S., Schaedelin, S., Manouchehrinia, A., Yaldizli, Ö., Maceski, A., Oechtering, J., Achtnichts, L., Conen, D., Derfuss, T., et al. (2022). Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol.* 21, 246–257. [https://doi.org/10.1016/S1474-4422\(22\)00009-6](https://doi.org/10.1016/S1474-4422(22)00009-6).
- Prinz, M., Masuda, T., Wheeler, M.A., and Quintana, F.J. (2021). Microglia and Central Nervous System-Associated Macrophages-From Origin to Disease Modulation. *Annu. Rev. Immunol.* 39, 251–277. <https://doi.org/10.1146/annurev-immunol-093019-110159>.
- Prinz, M., Jung, S., and Priller, J. (2019). Microglia Biology: One Century of Evolving Concepts. *Cell* 179, 292–311. <https://doi.org/10.1016/j.cell.2019.08.053>.
- Jordão, M.J.C., Sankowski, R., Brendecke, S.M., Sagar, Locatelli, G., Tai, Y.H., Tay, T.L., Schramm, E., Armbruster, S., Hagemeyer, N., et al. (2019). Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science* 363, eaat7554. <https://doi.org/10.1126/science.aat7554>.
- Sella, N., Verny, L., Uguzzoni, G., Affeldt, S., and Isambert, H. (2018). MIIC online: a web server to reconstruct causal or non-causal networks from

- non-perturbative data. *Bioinformatics* 34, 2311–2313. <https://doi.org/10.1093/bioinformatics/btx844>.
28. Kaiser, T., Dattero, J., Li, L., Chen, M., Jiang, M., Harrahil, A., Butovsky, O., and Feng, G. (2024). Transgenic Targeting of *Fcrls* Creates a Highly Efficient Constitutively Active Microglia Cre Line with Differentiated Specificity. *eNeuro* 11. ENEURO.0549-23.2024. <https://doi.org/10.1523/ENEURO.0549-23.2024>.
29. Sousa, C., Golebiewska, A., Poovathingal, S.K., Kaoma, T., Pires-Afonso, Y., Martina, S., Coowar, D., Azuaje, F., Skupin, A., Balling, R., et al. (2018). Single-cell transcriptomics reveals distinct inflammation-induced microglia signatures. *EMBO Rep.* 19, e46171. <https://doi.org/10.15252/embr.201846171>.
30. Silvín, A., Uderhardt, S., Piot, C., Da Mesquita, S., Yang, K., Geirsdottir, L., Mulder, K., Eyal, D., Liu, Z., Bridance, C., et al. (2022). Dual ontogeny of disease-associated microglia and disease inflammatory macrophages in aging and neurodegeneration. *Immunity* 55, 1448–1465.e6. <https://doi.org/10.1016/j.immuni.2022.07.004>.
31. Hammond, T.R., Dufort, C., Dissing-Olesen, L., Giera, S., Young, A., Wysoker, A., Walker, A.J., Gergits, F., Segel, M., Nemesh, J., et al. (2019). Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* 50, 253–271.e6. <https://doi.org/10.1016/j.immuni.2018.11.004>.
32. Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T.K., David, E., Baruch, K., Lara-Astaiso, D., Toth, B., et al. (2017). A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* 169, 1276–1290.e17. <https://doi.org/10.1016/j.cell.2017.05.018>.
33. Van Hove, H., Martens, L., Scheyltjens, I., De Vlaminc, K., Pombo Antunes, A.R., De Prijck, S., Vandamme, N., De Schepper, S., Van Isterdael, G., Scott, C.L., et al. (2019). A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat. Neurosci.* 22, 1021–1035. <https://doi.org/10.1038/s41593-019-0393-4>.
34. Haynes, S.E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M.E., Gan, W.B., and Julius, D. (2006). The P2Y₁₂ receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* 9, 1512–1519. <https://doi.org/10.1038/nn1805>.
35. Yamasaki, R., Lu, H., Butovsky, O., Ohno, N., Rietsch, A.M., Cialic, R., Wu, P.M., Doykan, C.E., Lin, J., Coteleur, A.C., et al. (2014). Differential roles of microglia and monocytes in the inflamed central nervous system. *J. Exp. Med.* 211, 1533–1549. <https://doi.org/10.1084/jem.20132477>.
36. Ajami, B., Bennett, J.L., Krieger, C., McNagny, K.M., and Rossi, F.M.V. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* 14, 1142–1149. <https://doi.org/10.1038/nn.2887>.
37. Mildner, A., Mack, M., Schmidt, H., Brück, W., Djukic, M., Zabel, M.D., Hille, A., Priller, J., and Prinz, M. (2009). CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain* 132, 2487–2500. <https://doi.org/10.1093/brain/awp144>.
38. Shemer, A., Scheyltjens, I., Frumer, G.R., Kim, J.S., Grozovski, J., Ayanaw, S., Dassa, B., Van Hove, H., Chappell-Maor, L., Boura-Halfon, S., et al. (2020). Interleukin-10 Prevents Pathological Microglia Hyperactivation following Peripheral Endotoxin Challenge. *Immunity* 53, 1033–1049.e7. <https://doi.org/10.1016/j.immuni.2020.09.018>.
39. Goldmann, T., Wieghofer, P., Jordão, M.J.C., Prutek, F., Hagemeyer, N., Frenzel, K., Amann, L., Staszewski, O., Kierdorf, K., Krueger, M., et al. (2016). Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat. Immunol.* 17, 797–805. <https://doi.org/10.1038/ni.3423>.
40. Cencioni, M.T., Mattosio, M., Magliozzi, R., Bar-Or, A., and Muraro, P.A. (2021). B cells in multiple sclerosis - from targeted depletion to immune reconstitution therapies. *Nat. Rev. Neurol.* 17, 399–414. <https://doi.org/10.1038/s41582-021-00498-5>.
41. Oliver, A.R., Lyon, G.M., and Ruddle, N.H. (2003). Rat and human myelin oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis by different mechanisms in C57BL/6 mice. *J. Immunol.* 171, 462–468. <https://doi.org/10.4049/jimmunol.171.1.462>.
42. Matsumoto, M., Baba, A., Yokota, T., Nishikawa, H., Ohkawa, Y., Kayama, H., Kallies, A., Nutt, S.L., Sakaguchi, S., Takeda, K., et al. (2014). Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. *Immunity* 41, 1040–1051. <https://doi.org/10.1016/j.immuni.2014.10.016>.
43. Rojas, O.L., Pröbstel, A.K., Porfili, E.A., Wang, A.A., Charabati, M., Sun, T., Lee, D.S.W., Galicia, G., Ramaglia, V., Ward, L.A., et al. (2019). Recirculating Intestinal IgA-Producing Cells Regulate Neuroinflammation via IL-10. *Cell* 177, 492–493. <https://doi.org/10.1016/j.cell.2019.03.037>.
44. Lino, A.C., Dang, V.D., Lampropoulou, V., Welle, A., Joedicke, J., Pohar, J., Simon, Q., Thalmens, J., Baures, A., Flüher, V., et al. (2018). LAG-3 Inhibitory Receptor Expression Identifies Immunosuppressive Natural Regulatory Plasma Cells. *Immunity* 49, 120–133.e9. <https://doi.org/10.1016/j.immuni.2018.06.007>.
45. Shalpour, S., Font-Burgada, J., Di Caro, G., Zhong, Z., Sanchez-Lopez, E., Dhar, D., Willmsky, G., Ammirante, M., Strasner, A., Hansel, D.E., et al. (2015). Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature* 521, 94–98. <https://doi.org/10.1038/nature14395>.
46. Shalpour, S., Lin, X.J., Bastian, I.N., Brain, J., Burt, A.D., Aksenov, A.A., Vrbanc, A.F., Li, W., Perkins, A., Matsutani, T., et al. (2017). Inflammation-induced IgA⁺ cells dismantle anti-liver cancer immunity. *Nature* 551, 340–345. <https://doi.org/10.1038/nature24302>.
47. Machado-Santos, J., Saji, E., Tröschner, A.R., Paunovic, M., Liblau, R., Gabriely, G., Bien, C.G., Bauer, J., and Lassmann, H. (2018). The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8⁺ T lymphocytes and B cells. *Brain* 141, 2066–2082. <https://doi.org/10.1093/brain/awy151>.
48. Pröbstel, A.K., Zhou, X., Baumann, R., Wischnewski, S., Kutza, M., Rojas, O.L., Sellrie, K., Bischof, A., Kim, K., Ramesh, A., et al. (2020). Gut microbiota-specific IgA⁺ B cells traffic to the CNS in active multiple sclerosis. *Sci. Immunol.* 5, eabc7191. <https://doi.org/10.1126/sciimmunol.abc7191>.
49. Borst, K., Dumas, A.A., and Prinz, M. (2021). Microglia: Immune and non-immune functions. *Immunity* 54, 2194–2208. <https://doi.org/10.1016/j.immuni.2021.09.014>.
50. Montilla, A., Zabala, A., Er-Lukowiak, M., Rissiek, B., Magnus, T., Rodriguez-Iglesias, N., Sierra, A., Matute, C., and Domercq, M. (2023). Microglia and meningeal macrophages depletion delays the onset of experimental autoimmune encephalomyelitis. *Cell Death Dis.* 14, 16. <https://doi.org/10.1038/s41419-023-05551-3>.
51. Sloka, S., Metz, L.M., Hader, W., Starreveld, Y., and Yong, V.W. (2013). Reduction of microglial activity in a model of multiple sclerosis by dipyrindamole. *J. Neuroinflamm.* 10, 89. <https://doi.org/10.1186/1742-2094-10-89>.
52. Goldmann, T., Wieghofer, P., Müller, P.F., Wolf, Y., Varol, D., Yona, S., Brendecke, S.M., Kierdorf, K., Staszewski, O., Datta, M., et al. (2013). A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nat. Neurosci.* 16, 1618–1626. <https://doi.org/10.1038/nn.3531>.
53. Heppner, F.L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hövelmeyer, N., Waisman, A., Rülcke, T., Prinz, M., Priller, J., et al. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat. Med.* 11, 146–152. <https://doi.org/10.1038/nm1177>.
54. Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., Beckers, L., O'Loughlin, E., Xu, Y., Fanek, Z., et al. (2017). The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* 47, 566–581.e9. <https://doi.org/10.1016/j.immuni.2017.08.008>.
55. Jie, Z., Ko, C.J., Wang, H., Xie, X., Li, Y., Gu, M., Zhu, L., Yang, J.Y., Gao, T., Ru, W., et al. (2021). Microglia promote autoimmune inflammation via the noncanonical NF- κ B pathway. *Sci. Adv.* 7, eab0609. <https://doi.org/10.1126/sciadv.ab0609>.

56. Bido, S., Nannoni, M., Muggeo, S., Gambarè, D., Ruffini, G., Bellini, E., Passeri, L., Iaia, S., Luoni, M., Provinciali, M., et al. (2024). Microglia-specific IL-10 gene delivery inhibits neuroinflammation and neurodegeneration in a mouse model of Parkinson's disease. *Sci. Transl. Med.* 16, eadm8563. <https://doi.org/10.1126/scitranslmed.adm8563>.
57. Navarro Negredo, P., You, J., Hauptschein, M., Schroer, A.B., Richard, D.J., Abhiraman, G.C., Tsai, A.P., Sun, E.D., Notarangelo, G., Ramirez-Matias, J., et al. (2026). Targeting immune cells in the aged brain reveals that engineered cytokine IL-10 enhances neurogenesis and improves cognition. *Immunity* 59, 458–476.e13. <https://doi.org/10.1016/j.immuni.2026.01.016>.
58. Cannella, B., Gao, Y.L., Brosnan, C., and Raine, C.S. (1996). IL-10 fails to abrogate experimental autoimmune encephalomyelitis. *J. Neurosci. Res.* 45, 735–746. [https://doi.org/10.1002/\(SICI\)1097-4547\(19960915\)45:6<735::AID-JNR10>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1097-4547(19960915)45:6<735::AID-JNR10>3.0.CO;2-V).
59. Kwilas, A.J., Grace, P.M., Serbedzija, P., Maier, S.F., and Watkins, L.R. (2015). The therapeutic potential of interleukin-10 in neuroimmune diseases. *Neuropharmacology* 96, 55–69. <https://doi.org/10.1016/j.neuropharm.2014.10.020>.
60. Zigmond, E., Bernshtein, B., Friedlander, G., Walker, C.R., Yona, S., Kim, K.W., Brenner, O., Krauthgamer, R., Varol, C., Müller, W., et al. (2014). Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. *Immunity* 40, 720–733. <https://doi.org/10.1016/j.immuni.2014.03.012>.
61. Redhu, N.S., Bakthavatchalu, V., Conaway, E.A., Shouval, D.S., Tsou, A., Goettel, J.A., Biswas, A., Wang, C., Field, M., Muller, W., et al. (2017). Macrophage dysfunction initiates colitis during weaning of infant mice lacking the interleukin-10 receptor. *eLife* 6, e27652. <https://doi.org/10.7554/eLife.27652>.
62. Wohlleben, G., Gray, D., and Schimpl, A. (1996). In vitro immunization of naive mouse B cells: establishment of IgM secreting hybridomas specific for soluble protein or hapten from B cells cultured on CD40 ligand transfected mouse fibroblasts. *Int. Immunol.* 8, 343–349. <https://doi.org/10.1093/intimm/8.3.343>.
63. Chiou, S.H., Winters, I.P., Wang, J., Naranjo, S., Dudgeon, C., Tamburini, F.B., Brady, J.J., Yang, D., Grüner, B.M., Chuang, C.H., et al. (2015). Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing. *Genes Dev.* 29, 1576–1585. <https://doi.org/10.1101/gad.264861.115>.
64. Chu, V.T., Graf, R., Wirtz, T., Weber, T., Favret, J., Li, X., Petsch, K., Tran, N.T., Sieweke, M.H., Berlek, C., et al. (2016). Efficient CRISPR-mediated mutagenesis in primary immune cells using CrispRGold and a C57BL/6 Cas9 transgenic mouse line. *Proc. Natl. Acad. Sci. USA* 113, 12514–12519. <https://doi.org/10.1073/pnas.1613884113>.
65. Efremova, M., Vento-Tormo, M., Teichmann, S.A., and Vento-Tormo, R. (2020). CellPhoneDB: inferring cell-cell communication from combined expression of multi-subunit ligand-receptor complexes. *Nat. Protoc.* 15, 1484–1506. <https://doi.org/10.1038/s41596-020-0292-x>.
66. Subramanian, A., Alperovich, M., Yang, Y., and Li, B. (2022). Biology-inspired data-driven quality control for scientific discovery in single-cell transcriptomics. *Genome Biol.* 23, 267. <https://doi.org/10.1186/s13059-022-02820-w>.
67. Badia-I-Mompel, P., Vélez Santiago, J., Braunger, J., Geiss, C., Dimitrov, D., Müller-Dott, S., Taus, P., Dugourd, A., Holland, C.H., Ramirez Flores, R.O., et al. (2022). decoupleR: ensemble of computational methods to infer biological activities from omics data. *Bioinform. Adv.* 2, vbac016. <https://doi.org/10.1093/bioadv/vbac016>.
68. Xie, Z., Bailey, A., Kuleshov, M.V., Clarke, D.J.B., Evangelista, J.E., Jenkins, S.L., Lachmann, A., Wojciechowski, M.L., Kropiwnicki, E., Jagodnik, K.M., et al. (2021). Gene Set Knowledge Discovery with Enrichr. *Curr. Protoc.* 1, e90. <https://doi.org/10.1002/cpz1.90>.
69. Korsunsky, I., Millard, N., Fan, J., Slowikowski, K., Zhang, F., Wei, K., Baglaenko, Y., Brenner, M., Loh, P.R., and Raychaudhuri, S. (2019). Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat. Methods* 16, 1289–1296. <https://doi.org/10.1038/s41592-019-0619-0>.
70. Melsted, P., Boeshaghi, A.S., Liu, L., Gao, F., Lu, L., Min, K.H.J., da Veiga Beltrame, E., Hjörleifsson, K.E., Gehring, J., and Pachter, L. (2021). Modular, efficient and constant-memory single-cell RNA-seq preprocessing. *Nat. Biotechnol.* 39, 813–818. <https://doi.org/10.1038/s41587-021-00870-2>.
71. Zappia, L., and Oshlack, A. (2018). Clustering trees: a visualization for evaluating clusterings at multiple resolutions. *GigaScience* 7, giy083. <https://doi.org/10.1093/gigascience/giy083>.
72. Cabeli, V., Verny, L., Sella, N., Uguzzoni, G., Verny, M., and Isambert, H. (2020). Learning clinical networks from medical records based on information estimates in mixed-type data. *PLoS Comput. Biol.* 16, e1007866. <https://doi.org/10.1371/journal.pcbi.1007866>.
73. Verny, L., Sella, N., Affeldt, S., Singh, P.P., and Isambert, H. (2017). Learning causal networks with latent variables from multivariate information in genomic data. *PLoS Comput. Biol.* 13, e1005662. <https://doi.org/10.1371/journal.pcbi.1005662>.
74. Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J.P., and Tamayo, P. (2015). The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst.* 1, 417–425. <https://doi.org/10.1016/j.cels.2015.12.004>.
75. Browaeys, R., Saelens, W., and Saeys, Y. (2020). NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat. Methods* 17, 159–162. <https://doi.org/10.1038/s41592-019-0667-5>.
76. Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
77. Wang, L. (2021). Single-cell normalization and association testing unifying CRISPR screen and gene co-expression analyses with Normalizr. *Nat. Commun.* 12, 6395. <https://doi.org/10.1038/s41467-021-26682-1>.
78. Vander Heiden, J.A., Yaari, G., Uduman, M., Stern, J.N.H., O'Connor, K.C., Hafner, D.A., Vigneault, F., and Kleinstein, S.H. (2014). pRESTO: a toolkit for processing high-throughput sequencing raw reads of lymphocyte receptor repertoires. *Bioinformatics* 30, 1930–1932. <https://doi.org/10.1093/bioinformatics/btu138>.
79. Wolf, F.A., Angerer, P., and Theis, F.J. (2018). SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol.* 19, 15. <https://doi.org/10.1186/s13059-017-1382-0>.
80. Germain, P.L., Lun, A., Garcia Meixide, C., Macnair, W., and Robinson, M.D. (2021). Doublet identification in single-cell sequencing data using scDbtFinder. *F1000Res* 10, 979. <https://doi.org/10.12688/f1000research.73600.2>.
81. Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W.M., 3rd, Zheng, S., Butler, A., Lee, M.J., Wilk, A.J., Darby, C., Zager, M., et al. (2021). Integrated analysis of multimodal single-cell data. *Cell* 184, 3573–3587.e29. <https://doi.org/10.1016/j.cell.2021.04.048>.
82. Satija, R., Farrell, J.A., Gennert, D., Schier, A.F., and Regev, A. (2015). Spatial reconstruction of single-cell gene expression data. *Nat. Biotechnol.* 33, 495–502. <https://doi.org/10.1038/nbt.3192>.
83. Amezquita, R.A., Lun, A.T.L., Becht, E., Carey, V.J., Carpp, L.N., Geistlinger, L., Marini, F., Rue-Albrecht, K., Risso, D., Soneson, C., et al. (2020). Orchestrating single-cell analysis with Bioconductor. *Nat. Methods* 17, 137–145. <https://doi.org/10.1038/s41592-019-0654-x>.
84. Young, M.D., and Behjati, S. (2020). SoupX removes ambient RNA contamination from droplet-based single-cell RNA sequencing data. *GigaScience* 9, giaa151. <https://doi.org/10.1093/gigascience/giaa151>.
85. Pettitt, S.J., Liang, Q., Rairdan, X.Y., Moran, J.L., Prosser, H.M., Beier, D.R., Lloyd, K.C., Bradley, A., and Skarnes, W.C. (2009). Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat. Methods* 6, 493–495. <https://doi.org/10.1038/nmeth.1342>.
86. Cossarizza, A., Chang, H.D., Radbruch, A., Abrignani, S., Addo, R., Akdis, M., Andrä, I., Andreatta, F., Annunziato, F., Arranz, E., et al. (2021). Guidelines for the use of flow cytometry and cell sorting in immunological

- studies (third edition). *Eur. J. Immunol.* **51**, 2708–3145. <https://doi.org/10.1002/eji.202170126>.
87. Samusik, N., Good, Z., Spitzer, M.H., Davis, K.L., and Nolan, G.P. (2016). Automated mapping of phenotype space with single-cell data. *Nat. Methods* **13**, 493–496. <https://doi.org/10.1038/nmeth.3863>.
88. Kimmel, J.C., Hwang, A.B., Scaramozza, A., Marshall, W.F., and Brack, A.S. (2020). Aging induces aberrant state transition kinetics in murine muscle stem cells. *Development* **147**, dev183855. <https://doi.org/10.1242/dev.183855>.
89. Sella, N., Hamy, A.S., Cabeli, V., Darrigues, L., Laé, M., Rey, F., and Isambert, H. (2022). Interactive exploration of a global clinical network from a large breast cancer cohort. *NPJ Digit. Med.* **5**, 113. <https://doi.org/10.1038/s41746-022-00647-0>.
90. Werner, A., Schäfer, S., Gleußner, N., Nimmerjahn, F., and Winkler, T.H. (2022). Determining immunoglobulin-specific B cell receptor repertoire of murine splenocytes by next-generation sequencing. *STAR Protoc.* **3**, 101277. <https://doi.org/10.1016/j.xpro.2022.101277>.
91. Gupta, N.T., Vander Heiden, J.A., Uduman, M., Gadala-Maria, D., Yaari, G., and Kleinstein, S.H. (2015). Change-O: a toolkit for analyzing large-scale B cell immunoglobulin repertoire sequencing data. *Bioinformatics* **31**, 3356–3358. <https://doi.org/10.1093/bioinformatics/btv359>.
92. Schwabenland, M., Brück, W., Priller, J., Stadelmann, C., Lassmann, H., and Prinz, M. (2021). Analyzing microglial phenotypes across neuropathologies: a practical guide. *Acta Neuropathol.* **142**, 923–936. <https://doi.org/10.1007/s00401-021-02370-8>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human CD14 FITC	BioLegend	Cat# 325604; RRID: AB_830677
Anti-human CD19 APC-Cy7	BioLegend	Cat# 302218; RRID: AB_314248
Anti-human CD3 Alexa Fluor 700	BioLegend	Cat# 300424; RRID: AB_493741
Anti-human CD4 PerCP-Cy5.5	BioLegend	Cat# 300530; RRID: AB_893322
Anti-human IgM	Santa Cruz Biotechnology	Cat# 109-006-129
Anti-human IL-10 PE	BD Biosciences	Cat# 559330; RRID: AB_397227
Anti-mouse APP	Millipore	Cat# MAB348-100UL; RRID: AB_2056583
Anti-mouse B220 Alexa Fluor 700	BioLegend	Cat# 103232; RRID: AB_493716
Anti-mouse CD11b Alexa Fluor 700	BioLegend	Cat# 101222; RRID: AB_493705
Anti-mouse CD11b APC-Cy7	BioLegend	Cat# 101226; RRID: AB_830642
Anti-mouse CD138 BV421	BioLegend	Cat# 142523; RRID: AB_11204257
Anti-mouse CD138 PerCP-Cy5.5	BioLegend	Cat# 142510; RRID: AB_2561601
Anti-mouse CD16/CD32	BD Biosciences	Cat# 553141; RRID: AB_394656
Anti-mouse CD19 Alexa Fluor 700	BioLegend	Cat# 115528; RRID: AB_493735
Anti-mouse CD19 APC-Cy7	BioLegend	Cat# 115530; RRID: AB_830707
Anti-mouse CD1d PE	BioLegend	Cat# 123510; RRID: AB_1236538
Anti-mouse CD1d PerCP-Cy5.5	BioLegend	Cat# 123514; RRID: AB_2073523
Anti-mouse CD21/35 Pacific Blue	BioLegend	Cat# 123414; RRID: AB_2085158
Anti-mouse CD23 APC	BioLegend	Cat# 101620; RRID: AB_2563438
Anti-mouse CD25	BioXcell	Cat# BE00014; RRID: AB_1107617
Anti-mouse CD25 Alexa Fluor 700	BioLegend	Cat# 102024; RRID: AB_493709
Anti-mouse CD25 PE	BioLegend	Cat# 101904; RRID: AB_312847
Anti-mouse CD3	BioRad	Cat# MCA1477; RRID: AB_321245
Anti-mouse CD4 Alexa Fluor 700	BioLegend	Cat# 100430; RRID: AB_493698
Anti-mouse CD4 Pacific Blue	BioLegend	Cat# 100428; RRID: AB_493647
Anti-mouse CD40	BioXcell	Cat# BE0016-2; RRID: AB_1107647
Anti-mouse CD40L PE-Cy7	BioLegend	Cat# 106512; RRID: AB_2563492
Anti-mouse CD45 BV711	BioLegend	Cat# 103147; RRID: AB_2564383
Anti-mouse CD45 BV785	BioLegend	Cat# 103149; RRID: AB_2564590
Anti-mouse CD45.1 APC	Life Technologies	Cat# 17-0453-82; RRID: AB_469398
Anti-mouse CD45.1 TotalSeq-B	BioLegend	Cat# 110755; RRID: AB_2819823
Anti-mouse CD45.2 PE-Cy7	BioLegend	Cat# 109830; RRID: AB_1186103
Anti-mouse CD68 PE-Cy7	BioLegend	Cat# 137016; RRID: AB_2562948
Anti-mouse CD81 biotin	BioLegend	Cat# 104903; RRID: AB_313138
Anti-mouse CD86 BV650	BioLegend	Cat# 105036; RRID: AB_11126147
Anti-mouse CD8a Alexa Fluor 700	BioLegend	Cat# 100730; RRID: AB_493703
Anti-mouse CX3CR1 BV421	BioLegend	Cat# 149023; RRID: AB_2565706
Anti-mouse FoxP3 FITC	Life Technologies	Cat# 11-5773-82; RRID: AB_465243
Anti-mouse GM-CSF FITC	BioLegend	Cat# 505404; RRID: AB_315380
Anti-mouse I-A/I-E BV510	BioLegend	Cat# 107636; RRID: AB_2561397
Anti-mouse IFN- γ APC	BD Biosciences	Cat# 554413; RRID: AB_398551
Anti-mouse Ig biotin	BD Pharmingen	Cat# 553999; RRID: AB_395196
Anti-mouse IgG1 biotin	BioLegend	Cat# 406604; RRID: AB_315063
Anti-mouse IgM	Santa Cruz Biotechnology	Cat# 115-006-075

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Anti-mouse IL-10 PE	BD Biosciences	Cat# 554467'; RRID: AB_395412
Anti-mouse IL-17a PerCP	BioLegend	Cat# 506944; RRID: AB_2566153
Anti-mouse IL-6 eFluor 450	Life Technologies	Cat# 48-7061-80; RRID: AB_2574102
Anti-mouse LAG-3 PE-Cy7	BioLegend	Cat# 125226; RRID: AB_2715763
Anti-mouse Ly-6C FITC	BioLegend	Cat# 128006; RRID: AB_1186134
Anti-mouse Ly-6G PerCP	BioLegend	Cat# 127654; RRID: AB_2616999
Anti-mouse MAC-3	BD Pharmingen	Cat# 553322; RRID: AB_394780
Anti-mouse P2RY12 PE	BioLegend	Cat# 848004; RRID: AB_2721645
Anti-mouse PD-1 APC	BioLegend	Cat# 135210; RRID: AB_2159183
Anti-mouse TNF Alexa Fluor 700	BioLegend	Cat# 506338; RRID: AB_2562918
PE Rat IgG2a κ Isotype Control	BD Pharmingen	Cat# 559317; RRID: AB_10050484
Anti-mouse Ly-6G PerCP/Cyanine5.5	Biolegend	Cat# 127616; RRID: AB_1877271
Anti-mouse Ly-6G Spark Blue 574 (Flexi-Fluor™)	Biolegend	Cat# 285146; RRID: AB_3662486
Anti-mouse CD11a APC/Fire 750	Biolegend	Cat# 162906; RRID: AB_3097443
Anti-mouse CD83 FITC	Biolegend	Cat# 121506; RRID: AB_572009
Anti-mouse Ly-6C Brilliant Violet 711	Biolegend	Cat# 128037; RRID: AB_2562630
Anti-mouse CD206 (MMR) PE/Cyanine7	Biolegend	Cat# 141719; RRID: AB_2562248
Anti-mouse CD11c BUV563	BD	Cat# 749040; RRID: AB_2873434
Anti-mouse F4/80 RB780	BD	Cat# 569223; RRID: AB_3684882
Anti-mouse Folate Receptor β (FR β) BUV615	BD	Cat# 759706; RRID: AB_3691778
Anti-Mouse CD115 (CSF-1R) BUV496	BD	Cat# 749974; RRID: AB_2874201
Anti-Mouse CD44 PE-Cy5	BD	Cat# 561861; RRID: AB_10896296
Anti-Mouse CD68 Brilliant Ultra Violet 805	Invitrogen	Cat# 368-0681-82; RRID: AB_3250963
Anti-mouse CD22 APC	Biolegend	Cat# 126110; RRID: AB_2561630
Anti-mouse CD83 Brilliant Violet 650	Biolegend	Cat# 121515; RRID: AB_11203713
Anti-mouse CD36 PerCP/Cyanine5.5	Biolegend	Cat# 102620; RRID: AB_2750188
Anti-mouse Ly-49D Biotin	Invitrogen	Cat# 13-5782-82; RRID: AB_10596515
Anti-mouse Ly-49D APC	Invitrogen	Cat# 17-5782-82; RRID: AB_10717290
Anti-mouse CD180 (RP105) pure-functional grade	Miltenyi biotec	Cat# 130-101-182; RRID: AB_2661341
Anti-mouse CD9 PE/Cyanine7	Biolegend	Cat# 124816; RRID: AB_2783075
InVivoMAb anti-mouse IL-10	Bio X Cell	Cat# BE0049, Clone: JES5-2A5; RRID: AB_1107696
InVivoMAb anti-mouse IL-10R (CD210)	Bio X Cell	Cat# BE0050, Clone: 1B1.3A; RRID: AB_1107611

Biological samples

Buffy coats from healthy donors	Charité Universitätsmedizin Berlin and Blutspendedienst Berlin,	Application numbers EA1/116/13
Human spleen	Hôpital intercommunal Créteil, France	N/A
PBMCs from CIS patients	University Hospital, Toulouse, France	NCT01981161/NCT00942214
PBMCs from healthy donors	University Hospital, Toulouse, France	reporting number DC-20152488
PBMCs from RRMS patients	University Hospital, Toulouse	NCT01981161/NCT00942214
PBMCs from RRMS patients	CRB HCL, Lyon, France	reporting number DC-2008-72
Whole blood from healthy donors	Etablissement Français du Sang, France	N/A

Chemicals, peptides, and recombinant proteins

0.5M EDTA, pH 8.0	Invitrogen	Cat# 15575-038
4% buffered formalin	SAV Liquid Production GmbH	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
β -mercaptoethanol	Life technologies	Cat# 31350010
Brefeldin A	Sigma	Cat# B5936
Cell Activation Cocktail	BioLegend	Cat# 423302
CFA	Sigma	Cat# #F5881
Collagenase IV	Gibco	Cat# 17104-019
CpG ODN 2006	Invivogen	Cat# tlrl-2006
CpG ODN1826	Invivogen	Cat# tlrl-1826
DMEM	Life technologies	Cat# 61965-059
DMSO	Sigma	Cat# D2650
DNAseI	Sigma	Cat# DN25
Eosin	Epredia	Cat# 6766007
Fetal bovine serum	Biowest	Cat# S1810-500
FVS575V	BD Biosciences	Cat# 565694
G418	Life technologies	Cat# 10131035
GolgiStop	BD Biosciences	Cat# 554724
Hemalaun	Morphisto	Cat #11769
HEPES	Life technologies	Cat# 15630056
human MOG ₁₋₁₂₅ protein	Anaspec	Cat# AS-55158-1000
LPS	Sigma	Cat# L2637
Luxol fast blue	Morphisto	Cat# 11125.00500
MACS BSA Stock Solution	Miltenyi Biotec	Cat# 130-091-376
Mitomycin C	Sigma	Cat# M5353
MOG ₃₅₋₅₅ peptide	Proteogenix	N/A, synthesis on demand
<i>Mycobacterium tuberculosis</i> H37RA	Difco Laboratories	Cat# 10218823
Non essential amino acids	Life technologies	Cat# 11140035
Penicillin/streptomycin	Life technologies	Cat# 15140-122
Percoll	GE Healthcare	Cat# 17-0891-02
R848	Sigma	Cat# SML0196-10MG
Retronectin	Takara	T100B
RBC lysis buffer	Sigma	Cat# R7757
Recombinant mouse BAFF	Home-made	N/A
Recombinant mouse IL-4	Home-made	N/A
RPMI	Life technologies	Cat# 61870044
Saponin	Sigma	Cat# 47035-50G-F
Sodium pyruvate	Life technologies	Cat# 11360070
Streptavidin BV711	BioLegend	Cat# 405241
Streptavidin BV785	BioLegend	Cat# 405249
Streptavidin HRP	BioLegend	Cat# 405210
Sunflower seed oil	Sigma	Cat# S5007
Tamoxifen	Sigma	Cat# T5648
TMB High Sensitivity Substrate Solution	BioLegend	Cat# 421501
Zombie UV Fixable Viability Kit	BioLegend	Cat# 423108
4-(acetylamino)-N-[4-(6-([4-(acetylamino)benzoyl]amino)-1H-benzimidazol-2-yl)phenyl]benzamide	Chembridge	Cat# 5181420; C3 in this paper
4-(acetylamino)-N-[4-(1H-benzimidazol-2-yl)phenyl]benzamide	Chembridge	Cat# 5770906; N1 is this paper
N-[4-(1H-1,3-benzodiazol-2-yl)phenyl]-3-acetamidobenzamide	UOrsy	Cat# PB30348097; N2 is this paper
N-[3-(1H-1,3-benzodiazol-2-yl)phenyl]-4-acetamidobenzamide	Enamine	Cat# Z52370542; N3 is this paper

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
N-(2-phenyl-1H-benzimidazol-5-yl)benzamide	Chembridge	Cat# 7192352; N4 is this paper
N-(2-phenyl-1H-1,3-benzodiazol-5-yl)acetamide	Otava	Cat# 4630668; N5 is this paper
4-amino-N-(4-(6-[(4-aminobenzoyl)amino]-1H-benzimidazol-2-yl)phenyl)benzamide	Chembridge	Cat# 5180084; N6 in this paper
N-(2-phenyl-1H-benzimidazol-6-yl)acetamide	Chembridge	Cat# 5248911; N7 in this paper
N-[2-(4-acetamidophenyl)-1H-1,3-benzodiazol-5-yl]acetamide	IBS	Cat# STOCK1S-10281; N8 is this paper
N-[4-(1H-benzimidazol-2-yl)phenyl]benzamide	Chembridge	Cat# 5577834; N9 is this paper
4-methyl-N-(4-(5-(4-methylbenzamido)-1H-benzo[d]imidazol-2-yl)phenyl)benzamide	Specs	Cat# AN-153/1159346; N10 is this paper
4-methoxy-N-(4-(5-(4-methoxybenzamido)-1H-benzo[d]imidazol-2-yl)phenyl)benzamide	Specs	Cat# AN-153/1159347; N11 is this paper
4-fluoro-N-(4-(5-(4-fluorobenzamido)-1H-benzo[d]imidazol-2-yl)phenyl)benzamide	Specs	Cat# AN-153/1159348; N12 is this paper
4-cyano-N-(4-(5-(4-cyanobenzamido)-1H-benzo[d]imidazol-2-yl)phenyl)benzamide	Specs	Cat# AN-153/1170708; N13 is this paper
2-phenyl-N-[2-[4-(2-phenylacetamido)phenyl]-1H-1,3-benzodiazol-5-yl]acetamide	Specs	Cat# STIK223482; N14 is this paper
N-(2-(2-fluorophenyl)-1-methyl-1H-benzo[d]imidazol-6-yl)-2-methoxybenzamide	This paper	1b
N-(2-(4-fluorophenyl)-1-methyl-1H-benzo[d]imidazol-6-yl)-2-methoxybenzamide	This paper	1f
2-methoxy-N-(1-methyl-2-(pyridin-2-yl)-1H-benzo[d]imidazol-6-yl)benzamide	This paper	1i
2-methoxy-N-(1-methyl-2-(pyridin-3-yl)-1H-benzo[d]imidazol-6-yl)benzamide	This paper	1j
2-methoxy-N-(1-methyl-2-(pyridin-4-yl)-1H-benzo[d]imidazol-6-yl)benzamide	This paper	1m
2-methoxy-N-(2-(4-methoxyphenyl)-1-methyl-1H-benzo[d]imidazol-6-yl)benzamide	This paper	1n
2-methoxy-N-(2-(pyridin-3-yl)imidazo[1,2-a]pyridin-6-yl)benzamide	This paper	2a
Critical commercial assays		
anti-mouse CD19 microbeads	Miltenyi Biotec	Cat# 130-121-301
B cell isolation kit II	Miltenyi Biotec	Cat# 130-091-151
Bio-Plex system for mouse IL-10	BioRad	Cat# Y6000000V3
CD43 microbeads	Miltenyi Biotec	Cat# 130-049-801
ELISA for mouse IL-10	BioTechne	Cat# DY419-05
ELISA for mouse IL-6	BioTechne	Cat# DY406-05
Foxp3/Transcription Factor Staining Buffer Set	Invitrogen	Cat# 00-5523-00
NF-light for CSF samples	UmanDiagnostics	Cat# 10-7001
RNeasy Mini Kit	Qiagen	Cat# 74106
CellTiter-Glo® 2.0 Cell Viability Assay	Promega	Cat# G9242
Deposited data		
Affymetrix array data	This paper	GEO: GSE220719
ATAC-seq data	This paper	GEO: GSE222227
scRNA-seq ex vivo data	This paper	GEO: GSE222061
scRNA-seq in vitro data	This paper	GEO: GSE219282
Experimental models: Cell lines		
CD40L-expressing L cells (L47)	Wohlleben et al. ⁶²	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
C57BL/6NRj	Janvier Labs, France	Strain #SC-C57N-F
CD45.1	Charles River, France	Strain# 494
<i>Cx3cr1^{creER};Il10ra^{fl/fl}</i>	Shemer et al. ³⁸	N/A
<i>H2^{-/-}</i>	The Jackson Laboratory	Strain #003584, RRID:IMSR_JAX:003584
<i>Il6.tdTomato</i>	This paper	N/A
<i>Il10.EGFP</i>	Neves et al. ⁴	N/A
<i>Il10.EGFP-Il6.tdTomato</i>	This paper	N/A
<i>H11cas9</i>	The Jackson Laboratory; Chiou et al. ⁶³	Strain #027650
<i>H11.Cas9-Il10.EGFP-Il6.tdTomato</i>	This paper	N/A
<i>Il10^{-/-}</i>	The Jackson Laboratory	Strain #002251, RRID:IMSR_JAX:002251
MD4	The Jackson Laboratory	Strain #002595, RRID:IMSR_JAX:002595
MD4xRAG ^{-/-}	This paper	N/A
<i>Prdm1.tdTomato</i>	This paper	N/A
<i>Rag2^{-/-}</i>	The Jackson Laboratory	Strain #008449, RRID:IMSR_JAX:008449
Recombinant DNA and oligonucleotides		
MSCV-pU6-(BbsI)-CcdB-(BbsI)-Pgk-Puro-T2A-BFP	Addgene ⁶⁴	# 86457
Scramble1	This paper	AACCCCGGCTGTCATCGCCG
Scramble2	This paper	GCGACAAGCGTTGAGTTTCGC
Scramble3	This paper	CCTATTAGTCCGGTTTAGTC
<i>Irf4</i> 1	This paper	ACCCGGACGACAATGGACAG
<i>Irf4</i> 2	This paper	CCGCATCCCGTGGAACACG
<i>Irf4</i> 3	This paper	ACGCGTCATGAACCTTGAGA
<i>Prdm1</i> 1	This paper	GACGAGAGTAGTCCCCCAG
<i>Prdm1</i> 2	This paper	GATGTTTCATCCCGTTCTGAC
Software and algorithms		
BioRender	BioRender	N/A
CellPhoneDB	Efremova et al. ⁶⁵	N/A
ddqcR library pipeline	Subramanian et al. ⁶⁶	N/A
DecoupleR	Badia-I-Mompel et al. ⁶⁷	N/A
EnrichR pipeline	Xie et al. ⁶⁸	N/A
FlowJo	FlowJo LLC, USA	N/A
ForeCyt™	Sartorius	N/A
GrapPad Prism	Dotmatics	N/A
Harmony pipeline	Korsunsky et al. ⁶⁹	N/A
Kallisto bustools workflow	Melsted et al. ⁷⁰	N/A
Louvain algorithm and clustree pipeline	Zappia et al. ⁷¹	N/A
MIIC network analysis; Brain - Microglia D+19 p.i.	This paper	https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_Brain_MG_D2
MIIC network analysis; Brain - Microglia D+31 p.i.	This paper	https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_Brain_MG_D14
MIIC network analysis; SC - Microglia D+19 p.i.	This paper	https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_SC_MG_D2
MIIC network analysis; SC - Microglia D+31 p.i.	This paper	https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_SC_MG_D14
MIIC network inference	Cabeli et al. ⁷² ; Verny et al. ⁷³	N/A
MSigDB hallmark gene set collection	Liberzon et al. ⁷⁴	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
nf-core atacseq pipeline	DOI: 10.5281/zenodo.2634132	N/A
NicheNET	Browaeys et al. ⁷⁵	N/A
NIH ImageJ	Schneider et al. ⁷⁶	N/A
Normaliser	Wang et al. ⁷⁷	N/A
Omiq	Dotmatics	N/A
pRESTO and Change-O tools	Heiden et al. ⁷⁸	N/A
Scanpy framework	Wolf et al. ⁷⁹	N/A
scDblFinder pipeline	Germain et al. ⁸⁰	N/A
Scripts for <i>in vitro</i> scRNA-seq data analysis	This paper	https://github.com/GChyzak/bcell-il10-mouse
Seurat objects	Hao et al. ⁸¹	N/A
Seurat workflow	Satija et al. ⁸²	N/A
SingleCellExperiment library	Amezquita et al. ⁸³	N/A
SoupX algorithm	Young et al. ⁸⁴	N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Mouse lines

C57BL/6NRj mice (Ly5.1⁺Ly5.2⁺) were used and referred as WT mice (Janvier Labs, France). *Il10*.EGFP mice were previously described.⁴ Heterozygous *Il10*.EGFP mice were used for the chemical compound screen. *Il10*.EGFP-*Il6*.tdTomato mice were generated by crossing *Il10*.EGFP and *Il6*.tdTomato mice. Homozygous mice at both alleles were used. These mice were crossed with *H11*.Cas9⁶³ to create *H11*.Cas9-*Il10*.EGFP-*Il6*.tdTomato mice. *Cx3cr1*^{creER}:*Il10ra*^{fl/fl} mice were a gift from Steffen Jung (Weizmann Institute of Science, Israel).³⁸ *Il10*^{-/-} (strain #002251), *H2*^{-/-} (strain #003584), MD4 (strain #002595), *H11*.Cas9 (strain #027650) and *Rag2*^{-/-} (strain #008449) mice were purchased from the Jackson Laboratory (Bar Harbor, USA). MD4 mice were backcrossed into *Rag2*^{-/-} background for at least 5 generations to generate MD4xRAG^{-/-} mice used hereafter. CD45.1 (Ly5.1⁺Ly5.2⁺) mice (strain #494) were purchased from Charles River (France) and bred in-house. All mice were bred and kept under specific and opportunistic pathogen free conditions. Experiments were reviewed and approved by appropriate institutional review committees and conducted according to German and French legislations.

Construction of *Il6*-IRES_Mer-iCre-Mer_GSG_2A_tdT mice

A 7,04kb genomic fragment (4,5kb for the 5' arm and 2,5kb for the 3' arm) encompassing terminal stop codon of the *Il6* gene was isolated from a BAC clone of C57BL/6 origin (clone n° RPCIB731M02121Q; <http://www.lifesciences.sourcebioscience.com>). An IRES_Mer-iCre-Mer_GSG_2A_tdT-Frt-NeoR-Frt cassette was introduced at twenty bases after the stop codon contained in the exon 5 of the *Il6* transcript and before the 3' untranslated region of the *Il6* gene. The targeting construct was abutted to a cassette coding for the diphtheria toxin fragment A expression cassette, and linearized with Pme1. JM8.F6 C57BL/6N ES cells⁸⁵ were electroporated with the targeting vector. After selection in G418, ES cell clones were screened for proper homologous recombination by DNA-PCR. A neomycin-specific probe was used to ensure that adventitious non-homologous recombination events had not occurred in the selected ES clones. Properly recombined ES cells were injected into BALB/c blastocysts. Germline transmission led to the excision of the Frt-Cre-NeoR-Frt cassette in the N1 mice due to the crossing between chimaeras (F0) and Flippase WT mice. *Il6*-Mer-iCre-Mer_GSG_2A_tdT mice were identified by PCR of tail DNA. The pair of primers named 1333-ScM_01_Fwd (5'ATAAAATAGTCCTTCCTACCC) and 1333-ScM_01_Rev (5'TTGTCAGTTCTTCGTAGAGA) amplifies a 263 bp band in case of the wild-type *Il6* allele, whereas the pairs of primers: 1333-ScM_03_Fwd (5'ATGGACGAGCTGTACAAGTA) and 1333-ScM_01_Rev (5'TTGTCAGTTCTTCGTAGAGA) amplified a 235 bp band in the case of the *Il6*-Mer-iCre-Mer_GSG_2A_tdT allele.

Construction of *Prdm1*-OST-STOP-Ires-MerCreMer-2A-tdT mice

A 6 kb genomic fragment (3kb for 5' arm and 3kb for de 3' arm) encompassing terminal stop codon of the *Prdm1* gene was isolated from a BAC clone of C57BL/6 origin (clone n° RPCIB731E17298Q; <http://www.lifesciences.sourcebioscience.com>). A Twin OST-STOP-Ires-MerCreMer-2A-tdT-Frt-NeoR-Frt cassette was introduced just before the 3' untranslated region of the *Prdm1* gene, with the twin-OST sequence fused in frame with that coded by exon 7 of *Prdm1*. To ensure the flexibility between *Prdm1* and Twin OST, a GSG linker was placed between *Prdm1* and OST tag. The targeting construct was abutted to a cassette coding for the diphtheria toxin fragment A expression cassette, and linearized with Pme1. JM8.F6 C57BL/6N ES cells⁸⁵ were electroporated with the targeting vector. After selection in G418, ES cell clones were screened for proper homologous recombination by DNA-PCR. A neomycin-specific probe was used to ensure that adventitious non-homologous recombination events had not occurred in the selected ES clones. Properly recombined ES cells were injected into BALB/c blastocysts. Germline transmission led to the

excision of the Frt-Cre-NeoR-Frt cassette in the N1 mice due to the crossing between chimaeras (F0) and Flippase WT mice. *Prdm1-OST-STOP_{ires}-MerCreMer-2A-tdT* mice were identified by PCR of tail DNA. The pair of primers named 1405-ScQ_01_Fwd (5'GCTGCCTTTGGAGGATCTGA) and 1405-ScQ_01_Rev (5'GCCTCATTATGCCCTGAGA) amplifies a 517 bp band in case of the wild-type *Prdm1* allele, whereas the pairs of primers: 1405-ScQ_02_Fwd (5'GCCACCACCTGTTCCGTGAC) and 1405-ScQ_01_Rev (5'GCCTCATTATGCCCTGAGA) amplified a 339 bp band in the case of the *Prdm1-OST-STOP_{ires}-MerCreMer-2A-tdT* allele.

Experimental autoimmune encephalomyelitis

To induce T cell- and B cell-dependent active experimental autoimmune encephalomyelitis (EAE), mice were immunized subcutaneously with 150μg of MOG₃₅₋₅₅ peptide (MEVGWYRSPFSRVVHLYRNGK, Proteogenix, France) or with 100μg human MOG₁₋₁₂₅ protein (Anaspec, #AS-55158-1000), respectively. Antigens were emulsified in CFA (Sigma, #F5881) supplemented with 400μg of *Mycobacterium tuberculosis* H37RA (Difco Laboratories, #10218823). Then, mice received intraperitoneal injections with 320ng pertussis toxin (Sigma, #P2980) at the time of immunization and 48 hours later. For experiments involving WT or Ly5.1 mice, immunization was performed in 8- to 10-week-old female mice. For experiments with *Cx3cr1^{creER}:Il10ra^{fl/fl}* mice, immunization was performed 6-8 weeks after tamoxifen (TAM, Sigma, #T5648) induction in male and female mice. Mice were monitored daily for the weight and based on the following clinical scores: 0=healthy; 1=flaccid tail; 2=impaired righting reflex and/or abnormal gait; 3=partial hind leg paralysis; 4=total hind leg paralysis; 5=hind leg paralysis with partial front leg paralysis. Mice sacrificed for ethical reasons (persistent weight loss >20%) were given a score 5.

CD25⁺ T cell depletion

CD25⁺ T cells were depleted *in vivo* by a single intravenous injection of anti-CD25 (clone PC-61.5.3, BioXcell, #BE0014) at 300μg/mouse 5 days prior to EAE induction. Depletion efficiency was monitored by flow cytometry the day of immunization, which showed a total loss of CD25⁺ T cells in blood, LNs and spleen (see Figure 3D).

Tamoxifen treatment

For induction of the Cre recombinase in *Cx3cr1^{creER}:Il10ra^{fl/fl}* mice, Tamoxifen (Sigma, #T5648) was dissolved in warm sunflower seed oil (Sigma, #S5007) and administrated to animals *via* gavage in four times, every other day. All animals were Tamoxifen-treated at 5-6 weeks of age. Each oral application consisted of 5mg of Tamoxifen at a concentration of 0.1 mg Tamoxifen/μl. Mice were immunized 6-8 weeks after Tamoxifen treatment.

Tissue collection

Bone marrow: femurs and tibias were collected and bones were flushed using U100-Insulin syringes followed by the removal of red blood cells using RBC lysis buffer (Sigma, #R7757). Blood was drawn at the submandibular level using a lancet and collected in heparin-coated tubes. Spleen, inguinal/draining lymph nodes were removed from mice and mechanically dissociated using a syringe-plunger followed by the removal of red blood cells using RBC lysis buffer. For CNS cell isolation, mice were anesthetized using a mixture of ketamine/xylazine and trans-cardiac perfusion was made with ice-cold PBS. Brain and spinal cord were removed and digested for 30 minutes at 37°C with Collagenase IV (1mg/ml, Gibco, #17104-019) and DNaseI (0.5mg/ml, Sigma, #DN25) in HBSS, followed by mechanical dissociation to obtain a single-cell suspension. Cells were isolated on a 30% Percoll gradient (GE Healthcare, #17-0891-02) by centrifugation for 20 min at 2000 rpm (without break).

Murine B cell cultures

Mouse B cells were isolated from spleens by negative selection using CD43 microbeads (Miltenyi Biotec, #130-049-801) and an automated magnetic-activated cell sorting (autoMACS) cell separator following manufacturer's instructions (Miltenyi Biotec). B cell purity after isolation was routinely >90%. B cells were isolated from both female and male mice.

For 3 day-culture protocols, mouse B cells were seeded at 5x10⁵ cells/well in 96-well plates at 37°C. RPMI was supplemented with 10% fetal bovine serum (Eurobio), 1% penicillin/streptomycin, 0.1% β-mercaptoethanol, 0.1% HEPES, 1% non-essential amino acids and 1% sodium pyruvate (all from Life Technologies) and subsequently referred as RPMI complete medium. B cells were stimulated in RPMI complete medium and supplemented with stimuli as mentioned in figures' legends.

For 7 day-culture protocols, B cells stimulated for 3 days were extensively washed with PBS and added on top of CD40L-expressing L47 stromal cells⁶² previously treated with Mitomycin C (Sigma, #M5353) using 400,000 L47 cells and 100,000 activated B cells per well in 6-well plates. The second 4-days culture step was done at 37°C in RPMI complete medium supplemented with IL-4/BAFF (1ng/ml and 100ng/ml, respectively), CpG/DMSO (1μg/ml) or CpG/N6 (1μg/ml and 0.25μM, respectively). For *in vivo* treatment before or during EAE induction, day 7-B cells were extensively washed, re-suspended in PBS at a concentration of 5x10⁷ cells/ml and 10⁷ cells/mouse were injected intravenously in the tail vein. For *in vivo* treatment, B cells were generated from the recipient mouse's corresponding sex.

For stability assessment cultures, cells harvested after 7 days of culture, as described above, were extensively washed with PBS and either left unstimulated (RPMI complete medium) or stimulated with TLR-agonists (1μg/ml each), anti-IgM (5μg/ml), anti-CD40 (10μg/ml, clone FGK4.5/FGK45), alone or in combination for 3 additional days at 37°C and analyzed daily.

For CRISPR-Cas9 editing of genes, naive B cells were isolated from the spleens of *H11.Cas9-*Il10*.EGFP-*Il6*.tdTomato* mice and preactivated on day -1 with anti-CD180 (RP105; 1 μ g/mL). On day 0, cells were transduced with retroviral particles produced with MSCV-pU6-(BbsI)-CcdB-(BbsI)-Pgk-Puro-T2A-BFP⁶⁴ (Addgene #86457) encoding single guide RNAs (sgRNAs) targeting the gene of interest. Retroviral transduction was performed on retronectin-coated, non-tissue culture-treated plates by centrifugation. After incubation for 3 h, cells were washed and cultured under iBreg cells-inducing conditions as described above. Puromycin was added to the culture on day1, and cells were analyzed on day 7 gating on BFP⁺ cells.

To examine the role of IL-10 in iBreg cells differentiation, naive B cells isolated from the spleens of WT or *H11.Cas9-*Il10*.EGFP-*Il6*.tdTomato* mice were cultured under iBreg cells-inducing conditions as described above, in the presence or absence of anti-IL-10 (10 μ g/mL) and anti-IL-10R (10 μ g/mL). Cells were harvested on day 7 and analyzed by flow cytometry.

Human B cell cultures

Human B cells were isolated from peripheral blood mononuclear cells (PBMCs) of healthy donors obtained from buffy coats or from heparin-coated tubes. For the screening of molecules inducing IL-10 secretion in human B cells (Figure S9B), B cells were isolated from spleens of patients undergoing a splenectomy for nonimmunological disease-related reasons (spherocytosis or sickle cell anemia). This study was conducted in accordance with the Declaration of Helsinki, with informed consent from each patient or the patient's family. B cells were isolated by negative selection using the B cell Isolation kit II (Miltenyi Biotec, #130-091-151) and automated cell separator autoMACS (Miltenyi Biotec) following the manufacturer's instructions. B cell purity after isolation was >90%. B cells were next seeded at 1×10^5 or 2×10^5 cells/well in 96-well plates with RPMI complete medium. Cells were stimulated at 37°C with 1.5 μ g/ml CpG ODN2006 and 2 μ g/ml anti-IgM for 48 hours. Chemical compounds were added at 0.25 μ M. For detection of IL-10 by flow cytometry, B cells were activated at 3×10^5 cells/well with CpG ODN2006 or at 1×10^5 cells/well with CpG ODN2006 and anti-IgM, in the presence of DMSO or compound 1n at 0.25 μ M (Figure S9C).

Clinically isolated syndrome and multiple sclerosis patients

Information about multiple sclerosis (MS) patient samples and associated controls used in this study are in Table S1. Part of the relapsing remitting (RR)MS samples and associated clinical data were obtained from NeuroBioTec (CRB HCL, Lyon, France, Biobank BB-0033-00046) with the MS collection reporting number DC-2008-72. Studies concerning clinically isolated syndrome patients and other RRMS patients were approved by the French South-West & Overseas ethical committee and were registered with the following identifiers: NCT01981161 and NCT00942214. For healthy donors, the study was approved by the French South-West & Overseas ethical committee and was registered at the French Ministry of Higher Education and Research (DC-20152488).

METHOD DETAILS

Flow cytometry staining procedures

Flow cytometry analyses were conducted according to the guidelines for the use of flow cytometry and cell sorting in immunological studies⁸⁶ using reagents described in STAR Methods. Fc receptors were systematically blocked with anti-mouse CD16/CD32 (clone 2.4G2, BD Biosciences) and viable cells were identified with the fixable viability stain FVS575V (BD Bioscience, #565694) or with the Zombie UV™ Fixable Viability Kit (BioLegend, #423108). Ki-67 expression was detected by intracellular staining using the eBioscience Fcγ3/Transcription Factor Staining Buffer Set (Invitrogen, #00-5523-00) following manufacturer's instructions. For analysis, doublets were excluded by plotting SSC-H vs FSC-A. Data were acquired with a BD LSR Fortessa SORP or a SONY ID7000™ spectral flow cytometer. Data were analyzed with FlowJo (FlowJo LLC, USA).

Intracellular cytokine staining

For T cell cytokine detection, total splenocytes were isolated and stimulated for 5 hours with 30 μ g/ml of MOG₃₅₋₅₅ in the presence of GolgiStop (BD Bioscience, #554724). For B cell cytokine detection, total splenocytes were isolated and stimulated for 5 hours with a mix of Cell Activation Cocktail (BioLegend, #423302) and 1 μ g/ml LPS from *E. coli* O55:B5 (Sigma, #L2637). Then, cells were collected, surface stained, fixed and intracellular staining performed using the eBioscience Fcγ3/Transcription Factor Staining Buffer Set (Invitrogen, #00-5523-00) following manufacturer's instructions.

For the detection of intracellular IL-10 expression in human B cells, after 3 days of culture, cells were restimulated for 5 hours in the presence of Brefeldin A (1 μ g/ml, Sigma, #B5936), GolgiStop (BD Bioscience, #554724) and Cell Activation Cocktail (BioLegend, #423302). Then, cells were collected, surface stained, fixed and intracellular staining performed using a home-made permeabilization buffer (0.5% Saponin, 2mM EDTA and 0.5% BSA in 1X PBS).

Fluorescence activated cell sorting

Mouse B cells were first enriched as described above using CD43 microbeads. Total mouse B cells (CD19⁺), follicular B cells (CD19⁺/CD21^{low}/CD23^{hi}/CD1d^{lo/+}) and marginal zone B cells (CD19⁺/CD21^{hi}/CD23^{low}/CD1d^{hi/+}) were purified by FACS using a BD Aria IIIu. Total mouse CD19⁺ blood B cells were directly purified by FACS after red blood cell lysis (Sigma, #R7757-100ML). Purity of sorted cells was >90%.

High-throughput flow cytometry screen

The 40,128-compound library was constituted at the Leibniz-Institut für Molekulare Pharmakologie im Forschungsverbund Berlin (FMP) and provided in 384-well plates with 352 compounds/plate. Analyses were performed in duplicates. Mouse B cells were isolated from heterozygous *Il10*.EGFP reporter mice using anti-CD19 microbeads (Miltenyi Biotec, #130-121-301). Purity was systematically >95%. 10^5 B cells were added per well of 384-well plate containing one compound/well at 6.7 μ M and LPS at 1.25 μ g/ml final concentration. On day 3, cells were resuspended in PBS/0.2% BSA supplemented with propidium iodide and measured by the High Throughput Flow Cytometry Screening System (IntelliCyt). Data were analyzed with ForeCyt™ software. 258 compounds were identified that significantly increased the percentage of *Il10*.EGFP⁺ B cells. These were re-tested using WT B cells for validation. Thus, WT B cells were stimulated in the presence or absence of compounds, and day 3 supernatants were assessed for IL-10 concentration by Bio-Plex system (Bio-Rad). This identified 14 compounds that increased IL-10 levels in culture supernatants, including C3 that was used in this study. The investigation of 9 analogs to C3 led to the identification of N6 that was used throughout this study.

Unsupervised flow cytometry data analysis for CNS CD45⁺CD11b⁺Ly6G[−] cells

CNS cells were stained using a panel comprising 20 antibodies (Table S4) and data acquired on a SONY ID7000™. Initial cleanup of flow cytometry data was performed in individual FCS files using FlowJo (v10.10). Debris, doublets, and dead cells were excluded using standard gating strategies based on FSC/SSC and viability dye exclusion. Moreover, CD45-negative cells and CD45⁺CD4⁺, CD45⁺CD8⁺, CD45⁺CD19⁺, as well as CD45^{hi}Ly6G^{hi} were also excluded by manual gating. Remaining CD45^{int/hi}CD11b⁺ populations from individual samples were concatenated, ensuring equal representation across experimental groups.

Uniform manifold approximation and projection (UMAP) algorithm was used for data dimensionality reduction. UMAP was computed using a nearest neighbors value of 15 and a minimum distance of 0.5, utilizing all extracellular and intracellular markers as input dimensions. Unsupervised clustering was then performed using the X-Shift algorithm to identify distinct cell subsets in an unbiased manner.⁸⁷ X-Shift was computed using a K-nearest neighbor (KNN) value of 20 to find "centroids" in high-dimensional space. The resulting X-Shift clusters were projected onto the UMAP embeddings to validate spatial coherence. The relative abundance (frequency) of each cluster was calculated as a percentage of total CD45⁺CD11b⁺Ly6G[−] cells. Clusters with frequency under 10% are not shown. A heatmap was generated based on the normalized Median Fluorescence Intensity (MFI) of all markers (Figure S7D). The difference in MFI is indicated for each marker by a blue/red scale that shows its relative expression across the 8 clusters.

Immunoassays

In Figures 1A and S9A, IL-10 concentrations in cell-free culture supernatants were determined using Bio-Plex kits (Bio-Rad) according to the manufacturer's instruction.

Cell-free supernatants from murine B cell cultures were otherwise used to determine IL-10 (Biotechne, #DY419-05) and IL-6 (Biotechne, #DY406-05) concentration by ELISA using manufacturer's instructions.

Cell-free supernatants from human B cell cultures were used to determine IL-10 (Biotechne, #DY217B-05) concentration by ELISA using manufacturer's instructions.

To detect anti-hMOG₁₋₁₂₅ IgG1 and anti-hMOG₁₋₁₂₅ Ig, hMOG₁₋₁₂₅ was coated on high binding ELISA plates at 5 μ g/ml overnight at 4°C. After blocking with PBS 1% BSA, serial dilutions (5-fold dilutions, from 1:20 to 1:2500) of mouse serum was applied. hMOG₁₋₁₂₅-specific IgG1 and hMOG₁₋₁₂₅-specific-Ig were detected using anti-mouse IgG1-biotin (800ng/ml) or anti-mouse Ig-biotin (333.33ng/ml), respectively. Streptavidin-HRP (1:2000 dilution) and TMB High Sensitivity Substrate Solution (BioLegend, #421501) were next used. Optical density (O.D.) was measured at 450nm and corrected by removing corresponding value obtained at 620nm.

Serum neurofilament light chain quantification

Mouse serum was prepared using standard procedure. Briefly, blood was drawn at the submandibular level in dry tubes, and, when clotted, serum was collected after centrifugation (2000g, 10 minutes, 4°C). Neurofilament light chain was quantified in the serum of mice using the NF-light™ ELISA for CSF samples (UmanDiagnostics, #10-7001) according to manufacturer's instructions.

scRNA-seq

For data related to Figures 1C–1E, S1E, S3A, and S3B, B cells were stimulated as described and viable CD19⁺CD45⁺ cells were sorted from cultures by FACS for sequencing. For data related to Figures 4 and S6, EAE was induced in WT mice. At day 17 post-EAE induction, mice were treated with 10^7 Ly5.1⁺ iBreg cells injected intravenously. 2 days (D+19 p.i.) and 14 days after treatment (D+31 p.i.), CNS (brain + spinal cord) was isolated from two batches of mice (four mice each). After tissue digestion, cells were stained with TotalSeq™-B anti-mouse CD45.1 (BioLegend, #110755) and viable CD45⁺ cells were sorted by FACS for library preparation. The scRNA-seq libraries were generated using Chromium Single Cell Next GEM 3' Library & Gel Bead Kit version 3.1 with Feature Barcode Technology (10X Genomics) according to the manufacturer's protocol. Gene expression (mRNA) and ADT libraries were generated. Briefly, cells were counted, diluted at 1,000 cells/ μ L in PBS + 0.04% BSA and 20,000 cells were loaded in the 10X chromium controller to generate single-cell gel beads in emulsion. After reverse transcription, gel beads in emulsion were disrupted. Barcoded complementary DNA was isolated and amplified by PCR. Following fragmentation, end repair and A-tailing, sample indexes were added during index PCR. The purified libraries were sequenced on a Novaseq (Illumina) with 28 cycles of read 1, 8 cycles of i7 index and 91 cycles of read 2, targeting a median depth of 45,000 reads per cell for gene expression and 5,000 reads per cell for

CITE-seq ADT libraries. scRNA-seq data for B cells activated *in vitro* have been deposited at NCBI GEO depository and are accessible with the accession number GSE219282, or using the link (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE219282>). scRNA-seq data for CNS CD45⁺ cells (brain and spinal cord) have been deposited at NCBI GEO depository and are accessible with the accession number GSE222061, or using the link (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE222061>).

scRNA-sequencing data processing for *in vitro* activated mouse B cells

The kallisto | bustools workflow⁷⁰ was used to pseudo align reads from the RNA libraries to the mouse reference genome (GRCm38, release 98) and generate molecular identifier (UMI) counts for each cellular barcode. The resulting cell × gene count matrices were imported to the Scanpy framework for subsequent analyses.⁷⁹ We manually set different thresholds for each sample on the number of counts, number of genes, and proportion of mitochondrial genes to eliminate low quality cells by looking at the corresponding quality metric distributions. We then excluded the genes *Gm42418* and *AY036118*, which were reported to overlap with an unannotated rRNA locus,⁸⁸ and all genes expressed in less than 50 cells in the whole dataset. The count matrix was then log-normalized with $\ln(1+x)$, and genes were scaled to unit variance and zero mean. For the UMAP visualizations, the two replicates for naive cells were merged.

For differential gene expression, and gene co-expression analyses, we excluded mitochondrial and ribosomal genes and used Normaliser.⁷⁷ *p*-values were corrected with the Benjamini–Hochberg procedure. To determine significant genes, we set a dot product or fold change threshold of 2 and determined a different *q*-value threshold for each sample. The *q*-value threshold was 10^{-n} where *n* is the lowest positive integer for which there were less than 25 *q*-values between 10^{-n} and $10^{-(n+1)}$. We then intersected the gene lists resulting from the differential gene expression or gene co-expression analyses to create the N6-gene signature. For UPR pathway activity inference, we used decoupleR⁶⁷ together with the MSigDB hallmark gene set collection.⁷⁴ The scripts written for the analysis of this dataset are available at: <https://github.com/GChyzak/bcell-il10-mouse>.

scRNA-sequencing data processing for CD45⁺ cells from brain and spinal cord of EAE mice

Sequencing reads were demultiplexed and aligned to the mouse reference genome (mm10, release 98, built from Ensembl sources) using the CellRanger Pipeline version 6.0.0. Filtered UMI count matrices were used for downstream analysis using the Seurat workflow.⁸² 41,000 cells were harvested from brain (respectively 37,000 for spinal cord) with expression of 32,000 features. Seurat objects⁸¹ were created with the particularity of including one CITE-seq column to identify CD45.1-positive cells. Removal of doublets was done according to scDblFinder pipeline⁸⁰ then straining of ambient RNAs was performed using default SoupX algorithm.⁸⁴ Manual quality control was achieved by manual subsetting after visualization of usual QC features: percentage of mitochondrial genes under 5%, RNA counts in each cell between 1,000 and 45,000, and more than 1,000 features detected for both brain and spinal cord. A data driven quality control was then accomplished using ddqcR library pipeline,⁶⁶ resulting in 30,000 (resp. 22,000) QC-controlled cells for the brain (resp. spinal cord). After that, normalization was performed using scRNA-seq assay-dependent of the theoretical size of cells normalization by SingleCellExperiment library⁸³ and using classic CLR normalization for the CITE-seq assay; both followed by scaling and PCA from Seurat library. The integration was achieved using default Harmony pipeline⁶⁹ and used solely for cell annotation (no use of integrated data for the rest of the analysis). After UMAP reduction (Seurat library), clustering of cells was obtained using the original Louvain algorithm and the clustree pipeline⁷¹; a resolution of 2 (resp. 2.5) was chosen for the brain (resp. spinal cord).

To identify cell populations, an extensive list of genes was used. At this resolution, microglia were considered as a unique cluster (with the exception of the daMG cluster expressing *Mki67* isolated from the other microglia) and were reprocessed and clustered individually later on to gain insights on functional states (UMAP reduction included (Figure 4A)). Subclusters of microglia were then annotated using marker genes of homeostatic and disease-associated microglia²⁶ (Figure 4B), marking the end of the pre-processing. Individual UMAPs are shown in Table S3, with the number of cells in each microglia states.

For the multiple differentially expressed genes (DEG) analysis, a Wilcoxon Rank Sum test with a Benjamini-Hochberg correction of the *p*-value was performed using FindMarkers from the Seurat library. Samples retrieved 2 days and 14 days post-iBreg cells injection were computed separately to avoid batch effect. *Gm10076A* and *AY036118* were excluded from the analysis as indicated above. In addition, a list of genes (*Igkc*, *Jchain*, *Ighm*, *Igkc1*, *Igkc2*, *Ighd*, *Mzb1*) was removed from analyses of non-B cells because they were identified as genes polluting the samples.

For CellPhoneDB analysis,⁶⁵ cell-cell interactions were inferred from combined expression of multi-subunit receptor-ligand complexes after conversion of the mouse genes to human genes using biomaRt. The CellPhoneDB pipeline was then run using the statistical analysis method and default parameters.

For the Gene Set Enrichment Analysis, genes of the subpopulation of interest were chosen from the DEG analysis (adjusted *p*-value < 0.05) if expressed (count > 0) by at least 60% of the cells of said subpopulation. The top 30 resulting genes (arranged by log2FC) were searched for enrichment using the EnrichR pipeline⁶⁸ (focusing on GO_Biological_Process_2015 database) and top 10 functions (arranged by adjusted *p*-value) were selected.

For MIIC network inference,^{72,73} genes of interest were selected considering results from previous analyses (DEG iBreg cells-treated vs non-treated, genes coding for ligand-receptor pairs highlighted by CellPhoneDB, genes of interest from bibliography) and were kept if expressed (count > 0) by at least 60% of the cells of one of the subpopulations. All ribosomal (*Rps* and *Rpl*) genes were averaged as *Rps_mean* and *Rpl_mean* in the networks. Treatment was added as a contextual variable. For microglia networks, daMG3-*Mki67* cluster was considered as separate and removed from the analysis. The MIIC algorithm was then ran on raw RNA

counts of selected variables using 0.01-skeleton confidence cut and 0.01-orientation confidence cut. Treatment subnetworks are shown in Figure 4E and full networks can be seen on the MIIC server website.^{27,89}

Brain - Microglia D+19 p.i.: https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_Brain_MG_D2

Brain - Microglia D+31 p.i.: https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_Brain_MG_D14

SC - Microglia D+19 p.i.: https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_SC_MG_D2

SC - Microglia D+31 p.i.: https://miic.curie.fr/job_results_showcase_2022.php?id=Necker_CNS_SC_MG_D14

For the NicheNET analysis,⁷⁵ their step-by-step Seurat analysis was performed on iBreg cell-microglia and iBreg cell-monocyte interactions from brain samples analyzed 2 days after iBreg cells injection (D+19 p.i.). Around 70 ligands were found active with 95% of them having Pearson score above 0.08 (default NicheNet threshold) for each analysis. Top ligands are shown. IL-10 interactions were highlighted with a different color for readability.

ATAC-sequencing

100,000 cells were harvested and nuclei isolated by incubating cells for 10 min on ice in nuclei extraction buffer (15 mM Tris-HCl, 60 mM KCl, 15 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine (free base), 1X cOmplete Protease Inhibitor Cocktail, 0.1% NP-40). Nuclei were centrifuged at 500g for 10 min at 4°C, the supernatant was discarded, the pellet was resuspended in 50μl ATAC reaction buffer (25μl 2x Illumina Tagment DNA buffer, 2.5μl Illumina Tagment DNA Enzyme 1, 22.5μl nuclease free water) and incubated at 37°C for 30 min. The reaction was stopped by adding 25mM EDTA. The tagmented DNA was purified (MinElute PCR Purification Kit, Qiagen) followed by PCR amplification using the NEBNext High-Fidelity 2X PCR Master Mix (New England Biolabs) and 0.2μM Nextera-Index-adapters (5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC [i5] TCG TCG GCA GCG TC-3' and 5'-CAA GCA GAA GAC GGC ATA CGA GAT [i7] GT CTC GTG GGC TCG G-3', Illumina) with 10 PCR cycles. The libraries were purified with 1x Agencourt AMPure XP (Beckman Coulter), the concentration was measured (Qbit HS kit) and the fragment distribution was checked on the Agilent 2100 Bioanalyzer before sequencing on the Illumina HiSeq 2500 with 2x50bp paired-end reads. The processing of the ATAC sequencing data, including the differential pairwise analyses, was done by the use of the nf-core atacseq pipeline (version 1.2.1; doi: 10.5281/zenodo.2634132). Differentially accessible regions (DARs) were defined by applying the following filters to the DESeq2 output: $FDR \leq 0.01$; $|FC| \geq 1$. The DARs were annotated to the closest genes by the use of the R package GenomicRanges. The annotation of genomic features was done by the use of the R package ChIPseeker. Genomic annotations of the DARs relate to the mouse genome version (mm10). ATAC coverage was set to the same range for all samples (0-3). ATAC-seq data for *in vitro* stimulated B cells have been deposited at NCBI GEO depository and are accessible with the accession number GSE222227, or using the link (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE222227>). The 7 DARs indicated for *Il10* (Figure 2B) correspond to DAR1: chr1: 130987200 – 130988103. DAR2: chr1: 130993664 – 130994178. DAR3: chr1: 131019577 – 131020171. DAR4: chr1: 131020589 – 131021890. DAR5: chr1: 131022527 – 131023266. DAR6: chr1: 131025088 – 131026376. DAR7: chr1: 131037954 – 131039084.

Immunoglobulin heavy chain repertoire sequencing

Immunoglobulin heavy chain repertoire sequencing was performed as described.⁹⁰ In brief, RNA was isolated with the RNeasy mini kit (Qiagen, #74106) from WT murine resting B cells and from B cells stimulated for 7 days with: CpG/DMSO, then L47/CpG/DMSO or CpG/N6, then L47/CpG/N6. Both first strand synthesis primers as well as reverse PCR primers contained UMI to correct for over-amplification bias. The pRESTO and Change-O tools were used for paired-end merging, quality filtering and VDJ annotation.⁷⁸ The diversity was calculated with the Alakazam package. The Argalaxy workflow was done as previously described.^{90,91} All these tools are part of the Immcantation/suite Docker image.

Affymetrix arrays

WT B cells were incubated with DMSO or N6 (0.25 μM) in RPMI complete medium for 6 hours and used for transcriptome analysis. cRNA were hybridized on Affymetrix MG 430 2.0 arrays, using standard Affymetrix protocol after quality control with Agilent 2100 Bioanalyzer and quantification with NanoDrop ND-1000 spectrophotometer. The significantly differentially regulated genes were detected using a t-test based R-script, with *p*-values adjusted using Benjamini-Hochberg procedure. In order to be selected in a comparison of two conditions, each Affy IDs had to fulfill the following criteria: (i) be present in at least 2 of the 3 arrays for at least one of the two conditions compared, (ii) to have a mean signal intensity higher than 50 in at least one of the two conditions, and (iii) to show an adjusted *p*-value <0.01 (t-test) in the comparison of the two conditions. The genes differentially expressed between control and N6-treated B cells were identified using t-test (*p*<0.01). Affymetrix gene array data for B cells treated with DMSO or N6 have been deposited at NCBI GEO depository and are accessible with the accession number GSE220719, or using the link (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE220719>)

Histology

Histology was performed after perfusion as described before.^{37,92} Spinal cords were removed on day 30 post-EAE induction and fixed in 4% buffered formalin (vol/vol). The spinal cord was cut in cross-sections. 5 cylinders of standing spinal cord were embedded into the FFPE block and sectioned within the FFPE block so that cross sections were created. Sections were stained with either hemalaun and eosin or LFB to assess the degree of demyelination. Immunohistochemistry was performed using MAC-3 (BD Pharmingen, clone M3/84) for macrophages and microglia, CD3 (BioRad, clone CD3-12) for T cells and APP (Millipore, clone 22C11) to

determine axonal damage. All regions were analyzed; white and grey matter. In total, 10 sections were analyzed per mouse, 5 mice per group; 1 mean = mean of 10 counted sections, presented mean = mean of 5 mice. Spinal cord sections were evaluated using a Keyence BZ-X810 device and the NIH ImageJ software.⁷⁶

Confocal imaging and three-dimensional reconstruction of microglia

Formalin-fixed, paraffin-embedded (FFPE) spinal cord tissues were sectioned at 25 μm and stained with an anti-Iba1 antibody and DAPI. Z-stack images were acquired using a confocal microscope (Leica SP8) with a step size of 0.33 μm . For each animal, a minimum of three and a maximum of five individual microglial cells were selected and reconstructed in three dimensions using Imaris software (Oxford Instruments).

Morphological parameters, including dendrite number, soma sphericity, and the ratio of covered volume to soma volume, were quantified using Imaris. Soma sphericity values ranged from 0 to 1, with higher values indicating increased spherical morphology. Quantitative analyses were performed in a blinded manner where applicable.

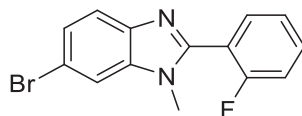
Chemical compound synthesis

Reagents were purchased as reagent-grade and used without further purification unless otherwise stated. Dioxane and Toluene were purchased as anhydrous-grade. Dimethylformamide (DMF) were freshly distilled before use. Analytical thin-layer chromatographies (TLC) were carried out on Macherey Nagel Alugram Xtra SIL G/UV254 plates using UV light ($\lambda = 254 \text{ nm}$) as visualizing agent. Flash chromatographies were carried out on puriFlash from Interchim equipped with packed silica cartridges from Macherey Nagel using a 100% DCM to 90/10 DCM/Ethanol (EtOH) gradient or a 100% Cyclohexane to 30/70 Cyclohexane/Ethyl acetate (EtOAc) gradient. Yields refer to chromatographically and spectroscopically pure compounds. NMR spectra were recorded on Bruker AV300 spectrometer at room temperature. Chemical shifts (δ), which are expressed in part per million (ppm), were determined relative to residual nondeuterated solvent as an internal reference respectively CDCl_3 and DMSO-d_6 ($(\text{CD}_3)_2\text{SO}$) (^{13}C NMR: $\delta = 77.23, 39.52 \text{ ppm}$; ^1H NMR: $\delta = 7.26; 2.50 \text{ ppm}$). Coupling constant(s) in hertz (Hz) were measured from one-dimensional spectra and multiplicities were abbreviated as following: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). The purity was determined by high performance liquid chromatography (HPLC) using an Alliance Waters system [Alliance Waters 2695 (pump) and Waters 2998 (Photodiode Array detector)] with a column Phenomenex Luna Omega C-18, 3 μm particle size (3,0 mm x 50 mm). Low-resolution mass spectrometry (ESI-MS) was recorded on a Waters Acquity QDa instrument (source voltage: 20 V). Syntheses under microwave irradiation are performed on a Discover microwave CEM equipped with an Explorer feeder.

General procedure A for N-methylbenzimidazole formation of compounds 6b, 6e, 6i–6j, 6l, and 6n

5-Bromo-N1-methylbenzene-1,2-diamine (**4**) (0.2 g, 1 mmol) is placed in anhydrous DMF (2 mL). Aldehyde **5** (1–2 eq) is then added in presence $\text{Na}_2\text{S}_2\text{O}_5$ (1–2 eq). The reaction mixture is irradiated under microwave at 110°C during 30 min or heated at 110°C during 4 to 18 hours. The reaction mixture is cooled and poured on ice to precipitate the compound **6**. Then the precipitate is washed by water and dried under vacuum overnight. Or, the reaction mixture is concentrated under reduced pressure and the residue is diluted with EtOAc. The organic phase is washed with water, decanted and dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue is purified by column chromatography on silica gel to give compound **6**.

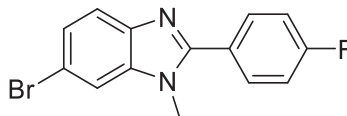
6-bromo-2-(2-fluorophenyl)-1-methyl-1H-benzo[d]imidazole (**6b**)



$\text{C}_{14}\text{H}_{10}\text{BrFN}_2$, MW: 305,15

Reaction was performed following general procedure **A**. Brown Solid (28%); ^1H NMR (300 MHz, DMSO-d_6) δ 7.96 (d, $J = 1.8 \text{ Hz}$, 1H), 7.78–7.60 (m, 3H), 7.53–7.37 (m, 3H), 3.71 (d, $J = 1.8 \text{ Hz}$, 3H); **MS** (ES+): $m/z = 305.2, 307.2 [\text{M}+\text{H}]^+$

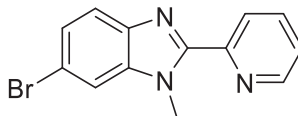
6-bromo-2-(4-fluorophenyl)-1-methyl-1H-benzo[d]imidazole (**6e**)



$\text{C}_{14}\text{H}_{10}\text{BrFN}_2$, MW: 305,15

Reaction was performed following general procedure **A**. Beige solid (90%); ^1H NMR (300 MHz, DMSO-d_6) δ 8.04 – 7.83 (m, 3H), 7.64 (d, $J = 8.5 \text{ Hz}$, 1H), 7.50 – 7.32 (m, 3H), 3.87 (s, 3H); **MS** (ES+): $m/z = 305.0, 307.0 [\text{M}+\text{H}]^+$

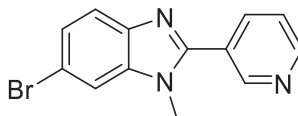
6-bromo-1-methyl-2-(pyridin-2-yl)-1H-benzo[d]imidazole (**6i**)



C₁₃H₁₀BrN₃, MW: 288,15

Reaction was performed following general procedure **A**. Yellow solid (66%); ¹H NMR (300 MHz, DMSO-d₆) δ 8.76 (d, J = 4.1 Hz, 1H), 8.30 (d, J = 7.9 Hz, 1H), 8.02 (td, J = 7.8, 1.8 Hz, 1H), 7.97 (d, J = 1.7 Hz, 1H), 7.68 (d, J = 8.6 Hz, 1H), 7.58-7.51 (m, 1H), 7.41 (dd, J = 8.6, 1.8 Hz, 1H), 4.22 (s, 3H); **MS** (ES⁺): m/z = 287.9, 289.9 [M+H]⁺

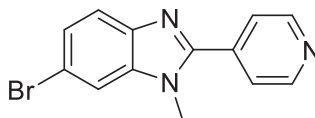
6-bromo-1-methyl-2-(pyridin-3-yl)-1H-benzo[d]imidazole (**6j**)



C₁₃H₁₀BrN₃, MW: 288,15

Reaction was performed following general procedure **A**. Pale purple solid (83%); ¹H NMR (300 MHz, DMSO-d₆) δ 9.05 (d, J = 1.5 Hz, 1H), 8.76 (dd, J = 4.8, 1.5 Hz, 1H), 8.39 – 8.20 (m, 1H), 7.99 (d, J = 1.6 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.62 (dd, J = 7.9, 4.8 Hz, 1H), 7.41 (dd, J = 8.6, 1.6 Hz, 1H), 3.91 (s, 3H); **MS** (ES⁺): m/z = 288.3, 290.3 [M+H]⁺

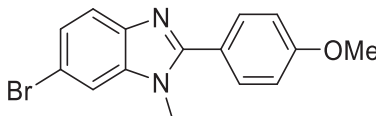
6-bromo-1-methyl-2-(pyridin-4-yl)-1H-benzo[d]imidazole (**6l**)



C₁₃H₁₀BrN₃, MW: 288,15

Reaction was performed following general procedure **A**. Orange solid (67%); ¹H NMR (300 MHz, DMSO-d₆) δ 8.79 (dd, J = 4.5, 1.5 Hz, 2H), 8.01 (d, J = 1.8 Hz, 1H), 7.88 (dd, J = 4.5, 1.5 Hz, 2H), 7.69 (d, J = 8.6 Hz, 1H), 7.42 (dd, J = 8.6, 1.8 Hz, 1H), 3.94 (s, 3H); **MS** (ES⁺): m/z = 287.9, 289.9 [M+H]⁺

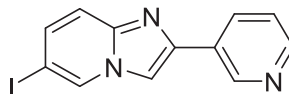
6-bromo-2-(4-methoxyphenyl)-1-methyl-1H-benzo[d]imidazole (**6n**)



C₁₅H₁₃BrN₂O MW: 317,19

Reaction was performed following general procedure **A**. Pink solid (71%); ¹H NMR (300 MHz, DMSO-d₆) δ 7.71 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.39 (dd, J = 8.5, 1.8 Hz, 1H), 7.05 (d, J = 8.7 Hz, 2H), 3.89 (s, 3H), 3.83 (s, 3H); **MS** (ES⁺): m/z = 316.9, 318.9 [M+H]⁺

6-iodo-2-(pyridin-3-yl)imidazo[1,2-a]pyridine (**10a**)



C₁₂H₈IN₃, MW: 321,12

2-amino-5-iodopyridine (**7**) (6.82 g, 31 mmol, 1.24 eq) is placed in absolute ethanol (50 mL). 2-bromo-1-(pyridin-3-yl)ethan-1-one hydrobromide (**8**) (5 g, 25 mmol, 1 eq) is then added in presence of NaHCO₃ (3.04 g, 36 mmol, 1.45 eq). The reaction mixture is heated at 80°C and stirred during 12 hours. The reaction is concentrated under reduced pressure and the resulting residue is purified by column chromatography on silica gel to give the compound **10a** in 8% yield (642 mg) as a yellow solid

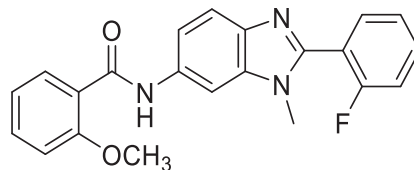
¹H NMR (300 MHz, DMSO-d₆) δ 9.15 (d, J = 1.5 Hz, 1H), 8.95 (s, 1H), 8.53 (dd, J = 4.7, 1.5 Hz, 1H), 8.44 (s, 1H), 8.30 (d, J = 8.1 Hz, 1H), 7.54 – 7.39 (m, 3H); **MS** (ES⁺): m/z = 322.1 [M+H]⁺

General procedure B for Palladium-catalyzed arylation of benzamide for 1b, 1f, 1i, 1j, 1m, and 1f

Compound **6** (50 mg, 1 eq) is placed in anhydrous dioxane (1 mL). Benzamide **9** (1-2 eq) is then added in presence of Xantphos (0.05 eq), Cs₂CO₃ (1.8 eq) and Tris(dibenzylideneacetone)dipalladium(0) (0.02 eq). The reaction mixture is heated at 150°C and stirred

during 15 to 72 hours in sealed tube under argon. The reaction mixture is concentrated under reduced pressure and the residue is diluted with DCM. The organic phase is washed with water, decanted and dried over magnesium sulfate, filtered and concentrated under reduced pressure. The resulting residue is dried purified by column chromatography on silica gel to give compound **1**.

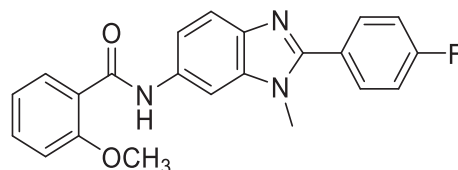
N-(2-(2-fluorophenyl)-1-methyl-1*H*-benzo[d]imidazol-6-yl)-2-methoxybenzamide (**1b**)



$C_{22}H_{18}FN_3O_2$, MW: 375,40

Reaction was performed following the general procedure **B**. Yellow solid (31%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.29 (s, 1H), 8.28 (s, 1H), 7.77–7.59 (m, 4H), 7.57–7.38 (m, 4H), 7.21 (d, J = 8.2 Hz, 1H), 7.09 (t, J = 7.2 Hz, 1H), 3.94 (s, 3H), 3.69 (d, J = 1.6 Hz, 3H); **MS** (ES $^+$): m/z = 376.4 [M+H] $^+$

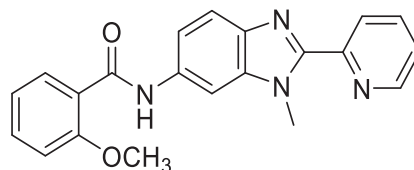
N-(2-(4-fluorophenyl)-1-methyl-1*H*-benzo[d]imidazol-6-yl)-2-methoxybenzamide (**1f**)



$C_{22}H_{18}FN_3O_2$, MW: 375,40

Reaction was performed following the general procedure **B**. Pale pink solid (15%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.27 (s, 1H), 8.26 (d, J = 1.6 Hz, 1H), 7.92 (dd, J = 8.8, 5.5 Hz, 2H), 7.70 (dd, J = 7.5, 1.6 Hz, 1H), 7.63 (d, J = 8.6 Hz, 1H), 7.56–7.48 (m, 1H), 7.47–7.37 (m, 3H), 7.21 (d, J = 8.3 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H); **MS** (ES $^+$): m/z = 376.4 [M+H] $^+$

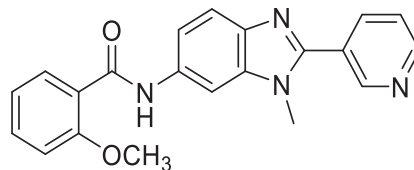
2-methoxy-*N*-(1-methyl-2-(pyridin-2-yl)-1*H*-benzo[d]imidazol-6-yl)benzamide (**1i**)



$C_{21}H_{18}N_4O_2$, MW: 358,40

Reaction was performed following the general procedure **B**. Orange solid (66%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.30 (s, 1H), 8.75 (d, J = 5.4 Hz, 1H), 8.36–8.24 (m, 2H), 8.00 (td, J = 7.7, 1.9 Hz, 1H), 7.74–7.63 (m, 2H), 7.57–7.46 (m, 2H), 7.44 (dd, J = 8.8, 1.9 Hz, 1H), 7.21 (d, J = 8.8 Hz, 1H), 7.09 (t, J = 7.2 Hz, 1H), 4.23 (s, 3H), 3.93 (s, 3H); **MS** (ES $^+$): m/z = 359.1 [M+H] $^+$

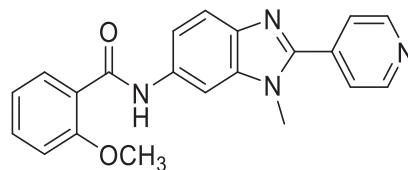
2-methoxy-*N*-(1-methyl-2-(pyridin-3-yl)-1*H*-benzo[d]imidazol-6-yl)benzamide (**1j**)



$C_{21}H_{18}N_4O_2$, MW: 358,40

Reaction was performed following the general procedure **B**. Beige solid (76%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.29 (s, 1H), 9.05 (d, J = 1.6 Hz, 1H), 8.74 (dd, J = 4.8, 1.6 Hz, 1H), 8.31–8.24 (m, 2H), 7.74–7.64 (m, 2H), 7.62 (dd, J = 7.9, 4.8 Hz, 1H), 7.57–7.48 (m, 1H), 7.44 (dd, J = 8.7, 1.8 Hz, 1H), 7.21 (d, J = 8.3 Hz, 1H), 7.09 (t, J = 7.4 Hz, 1H), 3.94 (s, 3H), 3.89 (s, 3H); **MS** (ES $^+$): m/z = 359.3 [M+H] $^+$; **HRMS** (ES $^+$): calculated for $C_{21}H_{19}N_4O_2^+$ 359.1508 found 359.1495.

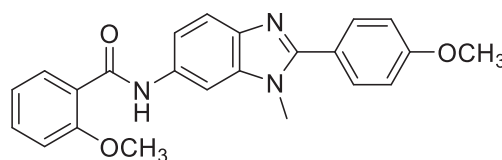
2-methoxy-N-(1-methyl-2-(pyridin-4-yl)-1H-benzo[d]imidazol-6-yl)benzamide (1m)



$C_{21}H_{18}N_4O_2$, MW: 358,40

Reaction was performed following the general procedure **B**. Yellow solid (72%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.31 (s, 1H), 8.78 (d, J = 6.0 Hz, 2H), 8.31 (s, 1H), 7.88 (d, J = 6.0 Hz, 2H), 7.74–7.63 (m, 2H), 7.59–7.47 (m, 1H), 7.45 (d, J = 7.1 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.09 (t, J = 7.3 Hz, 1H), 3.93 (s, 6H); **MS** (ES+): m/z = 359.1 $[M+H]^+$

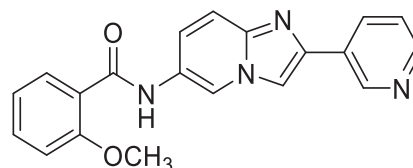
2-methoxy-N-(2-(4-methoxyphenyl)-1-methyl-1H-benzo[d]imidazol-6-yl)benzamide (1n)



$C_{23}H_{21}N_3O_3$, MW: 387,44

Reaction was performed following the general procedure **B**. White solid (80%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.24 (s, 1H), 8.22 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.70 (dd, J = 7.5, 1.8 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.52 (td, J = 7.9, 1.8 Hz, 1H), 7.39 (dd, J = 8.7, 1.9 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.16 – 7.05 (m, 3H), 3.94 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H); **MS** (ES+): m/z = 388.1 $[M+H]^+$

2-methoxy-N-(2-(pyridin-3-yl)imidazo[1,2-a]pyridin-6-yl)benzamide (2a)



$C_{20}H_{16}N_4O_2$, MW: 344,37

6-iodo-2-(pyridin-3-yl)imidazo[1,2-a]pyridine (**10a**) (50 mg, 0.16 mmol) is placed in dry toluene (1 mL). Benzamide **7** (1.2 eq., 0.19 mmol) is then added in presence of K_3PO_4 (66.3 mg, 0.32 mmol, 2 eq), copper(I) iodide (5.9 mg, 0.032 mmol, 0.2 eq), and trans-N,N'-dimethylcyclohexane-1,2-diamine (4.4 mg, 0.032 mmol, 0.2 eq). The reaction mixture is heated at 120°C and stirred during 48 hours in sealed tube under argon. The reaction mixture is diluted with DCM and filtrated through a celite pad. The filtrate is concentrated under reduced pressure. The resulting residue is purified by column chromatography on silica gel to give compound **2a**. Orange solid (19%); ^1H NMR (300 MHz, DMSO- d_6) δ 10.29 (s, 1H), 9.39 (s, 1H), 9.15 (s, 1H), 8.63 (s, 1H), 8.51 (d, J = 5.1 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.68–7.58 (m, 2H), 7.56–7.43 (m, 2H), 7.36 (d, J = 9.4 Hz, 1H), 7.21 (d, J = 8.6 Hz, 1H), 7.09 (t, J = 7.3 Hz, 1H), 3.91 (s, 3H); **MS** (ES+): m/z = 345.4 $[M+H]^+$; **HRMS** (ES+): calculated for $C_{20}H_{17}N_4O_2^+$ 345.1352 found 345.1357.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data presentation and statistical analyses were performed using GraphPad Prism (GraphPad Software, USA). Dispersion and precision of the data are indicated in figure legends. When two groups were compared, statistical analyses were performed with an unpaired Mann-Whitney test. Paired samples were analyzed with a Wilcoxon test. When more than two groups were compared, statistical analyses were performed with a Kruskal-Wallis test with Dunn's correction. When more than two groups with independent variables were compared, statistical analyses were performed with a two-way ANOVA followed by Tukey's multiple comparisons test. Level of significance were defined as followed: ns > 0.05, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. Data analysis and representation of scRNA-seq and ATAC-seq data are described in the corresponding sections.