Myeloid-derived suppressor cells control B cell accumulation in the central nervous system during autoimmunity

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Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) have been characterized in the context of malignancies. Here we show that PMN-MDSCs can restrain B cell accumulation during central nervous system (CNS) autoimmunity. Ly6G+ cells were recruited to the CNS during experimental autoimmune encephalomyelitis (EAE), interacted with B cells that produced the cytokines GM-CSF and interleukin-6 (IL-6), and acquired properties of PMN-MDSCs in the CNS in a manner dependent on the signal transducer STAT3. Depletion of Ly6G+ cells or dysfunction of Ly6G+ cells through conditional ablation of STAT3 led to the selective accumulation of GM-CSF-producing B cells in the CNS compartment, which in turn promoted an activated microglial phenotype and lack of recovery from EAE. The frequency of CD138+ B cells in the cerebrospinal fluid (CSF) of human subjects with multiple sclerosis was negatively correlated with the frequency of PMN-MDSCs in the CSF. Thus PMN-MDSCs might selectively control the accumulation and cytokine secretion of B cells in the inflamed CNS.

Suppressive myeloid cells were first described in tumor models accompanied by a strong leukemoid reaction. Based on surface markers in mice and humans, mononuclear (monocytic) myeloid-derived suppressor cells (M-MDSCs) and polymorphonuclear (granulocytic) MDCS (PMN-MDSCs) have been described. The surface lectin-type receptor LOX1, encoded by the OLR1 gene, is specifically expressed on PMN-MDSCs in humans. In mice, PMN-MDSCs are characterized as CD11b+Ly6G+Ly6Cint cells, which are also markers for bona fide neutrophils. However, because PMN-MDSCs are considered aberrantly activated neutrophils, the imprinting of distinct signaling pathways in CD11b+Ly6G+Ly6Cint cells can be used to detect MDCS in tissues of mice and humans. For instance, PMN-MDSCs respond to signals transduced by the transcription factor STAT3 for expansion and survival in situ, and robust activation of STAT3 is a hallmark of PMN-MDSCs and secures their functional phenotype. PMN-MDSCs strongly suppress CD8+ T cell responses against tumor cells. Less is known about the role of PMN-MDSCs in autoimmunity. PMN-MDSCs interact with B cells to inhibit the proliferation and differentiation of B cells in vitro and in vivo, or to induce regulatory B cells or IgA+ antibody–secreting cells. PMN-MDSCs also prevent the expansion of CD19+CD138+ B cells in an indirect manner by suppressing T follicular helper cell functions.

Despite the efficacy of B cell–depleting therapies in multiple sclerosis (MS), the function of B cells in MS is not well defined. B cells serve as precursors of antibody–secreting cells and provide cytokines, in particular interleukin-6 (IL-6), to promote germinal center reactions. B cells also present their cognate antigens to T cells and give T cells help. Compelling data supporting all of these functions have been reported in the context of central nervous system (CNS) autoimmunity in preclinical models. Circumstantial evidence from clinical trials suggests that B cell–depleting therapies in MS might mainly affect the antigen–presenting function of B cells. Notably, B cells that repopulate after B cell depletion tend to produce IL-10 instead of GM-CSF and contribute to sustained tolerance. Thus, in addition to their function as antigen–presenting cells, B cells might also exert a damaging function due to the secretion of cytokines. Finally, there is an ongoing debate about the structure and function of aggregates of B cells in the subarachnoid space. In particular, the initiation and severity of the chronic disease phase in MS is associated with the accumulation of these B cell aggregates. Therefore, B cells seem to facilitate a compartmentalized inflammatory process in the CNS that is disconnected from the systemic immune system.

Here, we sought to investigate the autoregulatory loops that control the recruitment, maintenance and function of B cells in the cerebrospinal fluid (CSF) and CNS parenchyma. Foxp3+ regulatory T cells (Treg cells) directly regulate some functions of B cells. However, in experimental autoimmune encephalomyelitis (EAE), depletion of Treg cells leads to an exacerbated disease course that...
depends on an enhanced effector T cell response, but that is independent of B cells, suggesting that Foxp3+ Treg cells dominantly regulate autoreactive T cells. Here we show that PMN-MDSCs inhibit the recruitment, local proliferation and cytokine secretion of CD138+ B cells in the CSF space and CNS parenchyma. Ly6G+ cells differentiated into PMN-MDSCs in the CNS in a gp130-STAT3-dependent manner.

### Results

PMN-MDSCs inversely correlate with CD138+ B cells in CSF.

Plasmablasts in the CSF are associated with disease activity in neuroinflammatory disorders including MS and neuromyelitis optica [19,20]. We tested whether an inverse correlation between the CSF plasmablast fraction and the fraction of any other immune cell population indicated a potential regulatory relationship. In the CSF of subjects with a first clinical episode suggestive of MS (clinically isolated syndrome (CIS)) or established MS, we found a robust negative correlation between the frequency of CD19+CD138+ B cells and the frequency of CD15+CD11b−LOX1+ myeloid cells (Fig. 1a), but not between the frequency of CD19+CD138- B cells and the frequency of LOX1- myeloid cells, total polymorphonuclear cells or any other immune cell subset in the CSF, including CD4+ T cells, CD8+ T cells, natural killer (NK) cells and CD14+ monocytes (Fig. 1a and Supplementary Fig. 1). CD15+CD11b−LOX1+ myeloid cells have been identified as PMN-MDSCs in humans [19]. In contrast to granulocytes, PMN-MDSCs are found in the low-density fraction in dual-density-gradient purification of peripheral blood cells and have a ‘monocytic’ appearance (Fig. 1b), which is in agreement with the fact that bona fide polymorphonuclear cells are not regularly found in CSF samples of people with MS. Upon coculture in vitro, CD15+CD11b−LOX1+ myeloid cells, but not LOX1- granulocytes, suppressed the proliferation of B cells stimulated with antibodies to CD40 and IL-4 (Fig. 1c), indicating that the negative correlation between the plasmablasts and PMN-MDSCs in the CSF of MS subjects could be due to the suppressive activity of PMN-MDSCs toward B cells.

Next, we evaluated the suitability of PMN-MDSCs as markers for disease activity in CIS and MS subjects. LOX1+ PMN-MDSCs, but not M-MDSCs, had higher levels in the peripheral blood of CIS and MS subjects than in that of healthy control subjects (Fig. 1d). This suggests that an inflammatory disease can trigger the appearance of PMN-MDSCs in the peripheral blood. Importantly, the frequency of LOX1+ PMN-MDSCs was significantly lower in MS subjects who had experienced a recent relapse than their frequency in stable MS subjects (Fig. 1c), a pattern that was observed regardless of whether the subjects were on disease-modifying therapy (Fig. 1f). Moreover, when we analyzed paired blood samples of MS subjects during relapse and at a follow-up visit (between 1 and 15 months later), the frequency of LOX1+ PMN-MDSCs increased in the MS subjects in whom inflammatory disease activity was entirely controlled and who were in a state known as ‘no evidence of disease activity’ (NEDA-3), as compared to subjects with ongoing disease activity (Supplementary Fig. 1). Thus, PMN-MDSCs were detectable in chronic CNS autoimmunity and their frequency in the peripheral blood decreased during episodes of active inflammatory disease, suggesting that PMN-MDSCs might be involved in regulating disease activity in MS.

Ly6G+ cells persist in CSF of mice during recovery from EAE.

To test the function of PMN-MDSCs in autoimmune neuroinflammation, we induced EAE in mice by immunization with myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 in complete Freund’s adjuvant (CFA); this treatment reflects many immune-mediated aspects of human MS [19]. We used Ly6G-tdTomato reporter mice, in which Ly6G+ cells can be tracked by expression of tdTomato (hereafter called Ly6Gcre/WT) [9], and analyzed Ly6G-tdTomato+ cells in the CNS, CSF and blood during EAE. We observed an increase in the fraction of Ly6G-tdTomato+ cells at disease onset (days 10–12) and a persisting population of Ly6G-tdTomato+ cells during the recovery phase (days 20–22) in the CSF compared to blood (Fig. 2a,b), concomitant with a second ‘peak’ of protein expression of the chemokine CXCL1 in the CSF (Fig. 2c). Phenotypically, the ‘monocytic’ appearance of Ly6G-tdTomato+ cells during the recovery phase was reminiscent of PMN-MDSCs, but very different from that of Ly6G-tdTomato+ cells collected from the peripheral immune compartment, which had segmented nuclei (Fig. 2d).

Inducible nitric oxide synthase (iNOS) was highly expressed and arginase activity strongly increased in Ly6G-tdTomato+ cells isolated from the CNS at recovery as compared to onset (Fig. 2e,f). Taken together, these data suggest that the Ly6G-tdTomato+ cells in the CNS during the recovery phase were phenotypically similar to PMN-MDSCs.

Ly6G+ cells acquire an MDSC-like transcriptome in the CNS.

To obtain a more comprehensive understanding of PMN-MDSCs during CNS autoimmunity, we performed RNA sequencing (RNAseq) analysis on Ly6G-tdTomato+ cells sorted from the spleen and CNS of Ly6Gcre/WT mice at onset (day 12) and during the recovery phase (day 22) of EAE. Principal component analysis segregated CNS-derived Ly6G-tdTomato+ cells from spleen-derived Ly6G-tdTomato+ cells (Fig. 3a). In particular, the transcriptome of CNS-derived Ly6G-tdTomato+ cells at disease onset seemed most distinct from all other subsets according to principal component 1 (PC1), and two MDSC hallmark genes, Nos2 and Arg1, were significantly upregulated in CNS-onset, but not CNS-recovery Ly6G-tdTomato+ cells compared to their expression in spleen Ly6G-tdTomato+ cells (Supplementary Fig. 2). Because Nos2 and Arg1 proteins were highly upregulated in CNS-recovery Ly6G-tdTomato+ cells compared to CNS-onset Ly6G-tdTomato+ cells (Fig. 2e,f), these data suggest that the transcriptome of CNS-onset Ly6G-tdTomato+ cells reflects the functional phenotype of CNS-recovery Ly6G-tdTomato+ cells, consistent with the delay imparted by RNA translation into protein. Moreover, in gene set enrichment analyses, a set of known human MDSC signature genes (such as PRDX1, ORL1, IRF8 and DOCK10) was enriched in samples with high PC1 and PC3 ranks (Fig. 3b and Supplementary Fig. 2). This indicates that the transcriptome of CNS-onset Ly6G-tdTomato+ cells was the most similar to a PMN-MDSC profile.

To further analyze the transcriptome of CNS-onset Ly6G-tdTomato+ cells, we identified 484 genes that were universally downregulated and 1,110 genes that were universally upregulated in CNS-onset Ly6G-tdTomato+ cells as compared to all other Ly6G-tdTomato+ cell subsets, respectively (Supplementary Table 1). Among the genes that were differentially downregulated in CNS-onset Ly6G-tdTomato+ cells, gene ontology terms including oxidoreductase activity, coenzyme binding and histone kinase activity were significantly overrepresented, whereas cytokine receptor binding, amide binding and enzyme inhibitor activity were significantly enriched in the set of genes universally upregulated in CNS-onset Ly6G-tdTomato+ cells (Fig. 3c, Supplementary Fig. 2 and Supplementary Tables 2 and 3). Notably, the mRNA for Olr1, the mouse homolog of human OLR1 (which encodes LOX1), was significantly upregulated in CNS-onset Ly6G-tdTomato+ cells compared to all other Ly6G-tdTomato+ populations (Supplementary Table 1). In summary, the PMN-MDSC signature was restricted to CNS Ly6G-tdTomato+ cells, whereas splenic Ly6G-tdTomato+ cells did not show an MDSC-like profile.

To test whether Ly6G+ cells acquired the MDSC profile in the inflamed CNS compartment, we transferred Ly6G-tdTomato+ cells isolated from the spleen of MOG(35–55) plus CFA-immunized CD45.2+ Ly6Gcre/WT mice into MOG(35–55) plus CFA-immunized congenic CD45.1+ host mice two days after onset of clinical signs...
of EAE and tracked CD45.2+Ly6G-tdTomato+ cells at days 1, 4 and 7 after transfer in the spleen and the CNS of host animals. Whereas transferred CD45.2+Ly6G-tdTomato+ cells disappeared from the peripheral immune compartment within two days, they accumulated and persisted in the CNS (Fig. 3d,e). Donor CD45.2+Ly6G-tdTomato+ cells were positive for the proliferation marker Ki67 in the inflamed host CNS, but not in the spleen, and CNS-derived Ly6G-tdTomato+ cells expressed high amounts of iNOS compared to Ly6G-tdTomato+ cells re-isolated from the spleen (Fig. 3f). These data indicate that Ly6G-tdTomato+ cells converted into PMN-MDSCs and accumulated in the CNS compartment.

Ly6G+ MDSCs prevent perpetuation of clinical signs of EAE. Given the overlap in phenotype between human PMN-MDSCs and murine Ly6G-tdTomato+ cells isolated from the CNS parenchyma, we tested whether the ablation or genetic loss of function of these cells in mice affected EAE progression. First, we depleted MDSCs in Ly6GCreWT mice with a monoclonal antibody to Ly6G starting at day 12, after onset of EAE. The depletion efficiency was around 90% in the blood and 80% in the CNS, as monitored by Ly6G-tdTomato expression (Supplementary Fig. 3). In contrast to mice treated with control antibody, Ly6G-tdTomato+ MDSC-depleted Ly6GCreWT mice did not recover from clinical signs of disease (Fig. 4a). Conversely,
expansion of Ly6G+ cells by administration of granulocyte colony-stimulating factor (G-CSF) as of day 12 led to faster and more complete recovery from clinical disease than in control-treated mice (Fig. 4b), suggesting that Ly6G+ cells were suppressive by the time EAE peaked (after day 16).

The numbers of CD11b+CD45+ macrophages and CD4+ T cells as well as the fraction of IL-17-, IFN-γ-, GM-CSF- and IL-10-producing CD4+ T cells and Foxp3+ Treg cells recovered from the CNS on day 21 were similar in control antibody–treated and Ly6G-ttdTomato+ MDSC-depleted Ly6gCre/WT mice (Supplementary Fig. 4). However, we detected a significantly greater (about three-fold) frequency and total number of CD19+ B cells in the spinal cord of Ly6G-ttdTomato+ MDSC-depleted Ly6gCre/WT mice as compared to control antibody–treated mice (Fig. 4c,e), and a lower frequency and number of CD19+ B cells in the spinal cord of mice treated with G-CSF as compared with Ly6gCre/WT mice treated with vehicle (5% glucose) (Fig. 4d–f). The frequencies of CD19+ B cells were inversely correlated with the frequencies of Ly6G+ cells in the CNS parenchyma of G-CSF-treated and control-treated Ly6gCre/WT mice at early disease recovery (day 21) (Fig. 4g). In addition, Ly6G-ttdTomato+ cells isolated from the CNS of Ly6gCre/WT mice at early recovery (day 20) suppressed the proliferation of B cells stimulated with antibodies to CD40 and IL-4 after in vitro coculture. In contrast, Ly6G-ttdTomato+ cells isolated from the CNS of Ly6gCre/WT mice at day 12 (EAE onset) and Ly6G-ttdTomato+ cells isolated from the spleen of Ly6gCre/WT mice at all disease stages did not suppress the proliferation of B cells in cocultures (Fig. 4h). These data suggest that Ly6G-ttdTomato+ cells modulated inflammation during EAE by inhibiting the proliferation of B cells in the CNS.

**STAT3 mediates Ly6G**+ cell conversion into MDSCs in the CNS. Myeloid bone marrow precursors generate PMN-MDSCs after stimulation with GM-CSF and IL-6 in a manner dependent on the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ)23. Based on quantitative PCR analysis, Il6st—which encodes gp130, the signaling subunit of the IL-6 receptor complex—was among the top genes upregulated in CNS-derived Ly6G-ttdTomato+ cells as compared to splenic Ly6G-ttdTomato+ cells (Fig. 5a). RNA-seq analysis on Ly6G-ttdTomato+ cells indicated that STAT3-targeted genes24 were highly enriched in CNS-onset Ly6G-ttdTomato+ cells compared to CNS-recovery and spleen Ly6G-ttdTomato+ cells (Fig. 5b). To test whether STAT3-dependent signaling determined the functional phenotype of Ly6G-ttdTomato+ cells in the CNS, we generated mice with a conditional deletion of STAT3 in Ly6G+ cells (Stat3Δlies hereafter) by crossing Ly6gCre/WT with Stat3Cre/WT mice. Stat3Δlies mice developed more severe EAE and did not recover from EAE as of day 20 (Fig. 5c), similarly to Ly6G-ttdTomato+ MDSC-depleted mice. Conditional deletion of gp130 in Ly6gCre/WT mice recapitated...
the phenotype of Stat3ΔινG mice (Supplementary Fig. 5). However, deletion of Il6ra, the IL-6-binding α-subunit of the IL-6 receptor complex, in Ly6G+ cells in Ly6GcreWT × Il6raflad mice did not lead to more severe EAE than in Ly6GcreWT control mice. These data suggest that the differentiation of PMN-MDSCs from Ly6G+ precursors in the CNS depends on gp130 and STAT3.

In addition to the prolonged clinical phenotype in Stat3ΔινG mice, we observed a greater frequency of CD19+ B cells in the CNS of Stat3ΔινG mice than in Ly6GcreWT control mice (Fig. 5d). The number and phenotype of T cells as well as the number of monocytes in the CNS of Stat3ΔινG mice were similar to those observed in Ly6GcreWT controls (Supplementary Fig. 6), suggesting that the STAT3-dependent activation of Ly6G+ MDSCs inhibited the accumulation of B cells but not other immune cells in the CNS during EAE. To address whether Ly6G+ MDSCs inhibited recruitment or expansion of B cells in the CNS, we measured the concentration of CSF chemokines required for B cell recruitment to the CNS by cytometric bead array. The amount of CXCL1 and CXCL13 protein in the...
CSF was higher in Stat3ΔLy6G mice than in Ly6gΔLy6C mice on day 24 (Fig. 5e), suggesting that greater recruitment might partially explain the higher levels of CD19+ B cells in the CNS of mice with STAT3-deficient Ly6G+ cells. Ly6G+ MDSCs suppress the proliferation of lymphocytes by a variety of mechanisms25,26. Coculture of CD19+ B cells and CD4+ T cells in the CD11b+ lymphoid compartment purified at early disease recovery (day 22) from spinal cord of Ly6gΔLy6C mice treated with control IgG or Ly6G antibody; representative plots of five mice from each group; gate on live CD45.2+CD11b+ cells. d, Flow-cytometry analysis of CD19+ B cells and CD4+ T cells in the CD11b+ lymphoid compartment purified at early disease recovery (day 21) from spinal cord of Ly6gΔLy6C mice treated or treated with G-CSF; representative plots of five mice from each group; gate on live CD45.2+CD11b+ cells. e, Total number of CD19+ B cells purified at early disease recovery (day 22) from spinal cord of Ly6gΔLy6C mice treated with control antibody (control, n = 5) or Ly6G antibody (Ly6g Ab, n = 5); symbols depict individual mice (bars, mean ± s.d.); Mann-Whitney U-test. **P < 0.01. f, Total number of CD19+ B cells purified at early disease recovery (day 21) from spinal cord of Ly6gΔLy6C mice control-treated or treated with G-CSF; symbols depict individual mice (bars, mean ± s.d.); Mann-Whitney U-test. **P < 0.01. g, Correlation of CD19+ B cell and Ly6G+ MDSC frequencies in the spinal cord of G-CSF-treated and control-treated Ly6gΔLy6C mice at early disease recovery (day 21); symbols depict individual mice; Spearman’s r; **P < 0.01. h, [3H] thymidine incorporation of CD40 antibody- and IL-4-stimulated CD19+ B cells and CD4+ T cells in the CD11b– lymphoid compartment purified at early disease recovery (day 22) from spinal cord of Ly6gΔLy6C mice treated with control IgG or Ly6G antibody; representative plots of five mice from each group; Kruskal-Wallis test with Dunn’s post-test; *P < 0.05.

Ly6G+ B cells interact with B cells in the CNS. Next, we characterized B cells in the CNS in the presence or absence of Ly6G+tgTomato+ cells. The B cells in the CNS of Stat3ΔLy6C mice or in Ly6gΔLy6C×tgTomato+ MDSC-depleted Ly6gΔLy6C×tgTomato+ mice with EAE were IgM+CD21+CD35+ B cells. We observed 30% greater fractions of activated CD23hi B cells in these mice as compared to wild-type EAE mice (Fig. 6a and Supplementary Fig. 7). There was no difference in the percentage of CD138 plasma cells in either the spleen or the CNS of Stat3ΔLy6C×tgTomato+ mice compared to that in Ly6gΔLy6C×tgTomato+ controls (Fig. 6b). CD23 is an activation marker of B cells, and CD23hi B cells in the CNS coexpressed IL-6 and GM-CSF (Fig. 6c). Neither CD1hiCD5+ B cells, which have regulatory properties27, nor unconventional B1 cells, which are CD123low (ref. 28), contributed to the greater numbers of CD19+ B cells purified at early disease recovery (day 21) from spinal cord of Ly6gΔLy6C mice control-treated or treated with G-CSF; symbols depict individual mice (bars, mean ± s.d.); Mann-Whitney U-test. **P < 0.01. 

In addition to a few cells scattered in the parenchyma, the majority of CD19hiB220+ B cells in Stat3ΔLy6C mice were clustered together in the meninges (Fig. 3i and Supplementary Fig. 7). Together, these data suggest that Ly6G+ MDSCs suppressed B cell recruitment and proliferation in the meningeal compartment in wild-type mice during EAE.
the meninges and superficial CNS parenchyma in Stat3^S/AaGc^ mice as compared to Ly6G^Cre/WT^ mice, which essentially lacked meningeal B cell infiltrates (Fig. 6f.h). This suggests a direct interaction between B cells and Ly6G^+^ cells in the meningeal compartment. To test whether direct interaction between B cells and Ly6G^+^ cells led to activation of STAT3 in Ly6G^+^ cells, splenic Ly6G^+^ cells from C57BL/6 mice immunized with MOG(35–55) plus CFA were isolated on day 8 and incubated with MOG(35–55) or MOG-specific T cells in direct contact with the B cells, but not in pSTAT3, was higher in Ly6G^+^ cells in response to IL-6–IL-6Rα than in response to equimolar amounts of IL-6 (Fig. 6l), consistent with the lower expression of IL-6Rα on the cell membrane of activated Ly6G^+^ cells. Notably, when Ly6G^+^ cells isolated from the spleen of MOG(35–55) plus CFA-immunized wild-type mice on day 8 were cocultured with MOG-specific B cell receptor transgenic CD19^+^ B cells isolated from the spleen of MOG-specific Ig heavy-chain knock-in mice (also referred to as TH mice) that were activated to produce IL-6 in vitro by coculture with MOG-specific T cell receptor transgenic T cells and MOG protein, we detected an increase in pSTAT3 only in Ly6G^+^ cells in direct contact with the B cells, but

**Fig. 5** | Ly6G^+^ cells differentiate into MDSCs in the CNS in a STAT3-dependent manner. **a.** Quantitative RT-PCR analysis of Il6st mRNA (which encodes gp130) in Ly6G^+^ cells purified from naive bone marrow (BM naive, n = 3), naive spleen (spleen naive, n = 4), and from spleen (spleen EAE, n = 4) and CNS (CNS EAE, n = 4) of EAE mice (day 17 after immunization); results are normalized relative to Ly6G^+^ cells purified from naive spleen; symbols depict individual mice (bars, mean ± s.d.); one-way ANOVA with Tukey’s post test; ****P < 0.0001. **b.** Gene set enrichment analysis, testing a set of STAT3-targeted genes on subsets of Ly6G^+^ cells. FC, fold change. **c.** EAE disease course in Ly6G^Cre/WT^ (n = 10) and Stat3^S/AaGc^ (n = 14) mice; symbols depict mean ± s.d.; two-way ANOVA with Bonferroni’s multiple comparison test; **P < 0.01; representative disease course out of three independent experiments. **d.** Flow-cytometry analysis of CD19^+^ B cells and CD4^+^ T cells in the live CD45.2^+^CD11b^–^ lymphoid compartment in the spinal cord of Ly6G^Cre/WT^ and Stat3^S/AaGc^ mice at early recovery (day 24); representative plots of eight mice in each group. **e.** Analysis of CXCL1 and CXCL13 protein levels in the CSF of Ly6G^Cre/WT^ (n = 5) and Stat3^S/AaGc^ (n = 6) mice at early disease recovery (day 24); symbols depict individual mice (bars, mean ± s.d.); unpaired Student’s t-test; **P < 0.01. **f.** $[^{3}H]$thymidine incorporation of CD40 antibody– and IL-4-stimulated CD19^+^ splenic B cells from the CNS of Ly6G^Cre/WT^ (n = 5) and Stat3^S/AaGc^ (n = 6) mice at early recovery (day 24); symbols depict individual mice (bars, mean ± s.d.); unpaired Student’s t-test; **P < 0.05. **g.** Flow-cytometry analysis of intracellular Ki67 in CD3^+^ T cells and CD19^+^ B cells among CNS CD45^+^ leukocytes in MOG(35–55) plus CFA-immunized wild-type mice on day 8. **h.** FoxP3^+^ Tregs (3A^S/AaGc^ in green) and pSTAT3^+^ EAE mice (i) at early recovery (day 23); ×20 magnification, scale bar, 100 μm; representative of five mice in each group.
not in Ly6G+ cells separated from the B cells in a transwell chamber (Fig. 6j). The increase in pSTAT3 in Ly6G+ cells was partly reversible by blockade of IL-6Rα with a neutralizing antibody to IL-6Rα (Fig. 6j). These data suggest that direct cell contact between Ly6G+ cells and B cells was required to activate STAT3 in Ly6G+ cells, and that such interactions in the CNS might drive the conversion of Ly6G+ cells into MDSCs, which in turn controls the activation of B cells in the CNS.

B cells in CNS prevent recovery from EAE in Stat3ΔLy6G mice.
To test whether the diminished capacity to recover from EAE of Stat3ΔLy6G mice was due to the higher frequency of activated CD23+IgM+ B cells in the CNS of these mice compared to Ly6GΔWT mice, we depleted the B cells by intravenous (i.v.) administration of an antibody to CD20. Intrauterine treatment with an antibody to CD20 every five days after EAE onset (day 12) led to significant depletion of B cells in the brain and spinal cord of Stat3ΔLy6G mice to numbers below those observed in wild-type EAE mice (Fig. 7a). In addition, the percentage of GM-CSF+ B cells in CD20 antibody–treated EAE Stat3ΔLy6G mice was similar to that found in wild-type EAE mice (Fig. 7b). Treatment with CD20 antibody led to a more severe disease course of MOG(35–55)-induced EAE in wild-type mice compared to control antibody–treated wild-type mice (Fig. 7c), consistent with previous reports. In contrast, administration of the CD20 antibodies ameliorated clinical disease severity in MOG(35–55)-induced EAE in Stat3ΔLy6G mice as compared to control antibody–treated Stat3ΔLy6G mice (Fig. 7d), indicating that B cells are responsible for the more severe EAE and chronic disease phenotype in the Stat3ΔLy6G mice.

Activated microglia have been associated with chronic inflammation in the CNS. RNA-seq data from microglia isolated from a chronic EAE model, as well as from experimental models of amyotrophic lateral sclerosis and Alzheimer’s disease, have been used to define a ‘neurotoxic’ microglia profile. As such, we tested whether Ly6G+ neutrophils differentiate into MDSCs in the CNS of mice with EAE during the recovery stage. Our analyses indicate that Ly6G+ neutrophils differentiate into MDSCs in the CNS of mice with EAE in the absence of functional MDSCs in the CNS are associated with tissue-destructive microglia activation.

Discussion
Here we show that immunoregulatory PMN-MDSCs are found as LOX1+ MDSCs in the CSF of MS subjects and as Ly6G+ MDSCs in the CNS of mice with EAE during the recovery stage. Our analyses indicate that Ly6G+ neutrophils differentiate into MDSCs in the CNS of wild-type mice in a STAT3-dependent manner and in turn control the accumulation and activation of B cells in this compartment. Loss of MDSCs leads to activation of B cells in the CNS, which—in part by secretion of GM-CSF—contributes to the establishment of compartmentalized inflammation in the CNS. B cells might be inducing an MDSC phenotype in a subset of neutrophils in the CNS, establishing a negative feedback loop that prevents perpetuation of inflammation in the CNS.

Bona fide neutrophils are short-lived. Their half-life is in the range of 5–10 h in the systemic compartment, and they must be constantly replenished by the bone marrow. Even though genetic tools for the study of neutrophils have been limited, the role of neutrophils in EAE has been addressed and they are considered to exert a proinflammatory function either by activating endothelial cells at the blood-brain-barrier for the enhanced recruitment of inflammatory cells, by inducing the maturation of antigen-presenting cells in the CNS, or through direct tissue destruction. However, because autoimmune attacks of the CNS in humans are rarely hyperacute, the proinflammatory role of neutrophils might be limited. Rather, myeloid cells of neutrophil origin that reside in parenchymal tissues for up to 6 days (ref. 39) may be more relevant to the disease course of CNS autoimmunity.

Analyses of immune cell population dynamics in the CSF and CNS parenchyma of mice during EAE revealed a persistent population of Ly6G+ cells at the beginning of the recovery stage. Because CXCL1 was also upregulated in the CNS at this stage, it is probable that various chemokine cues attract Ly6G+ precursors into the CSF space through the chemokine receptor CXCR2 expressed on Ly6G+ cells. Murine CXCL1 is the functional homolog of human chemokines CXCL1 and CXCL8, which are detected in the CSF of people with MS, and CXCL1 has been described as a hallmark effector molecule of Tg17 cell–driven autoimmunity in the CNS during EAE. Ly6G+ cells recruited to the CSF space during EAE differentiated into MDSCs in a STAT3-dependent manner. Notably,
hyperactivation of STAT3 in LysM+ cells, which also make up a sub-
set of neutrophils, leads to expansion of MDSCs in the colon and
protection from experimental colitis42. Although the differentia-
tion of Ly6G+ cells into MDSCs in the CNS required gp130 and STAT3,
it remains to be determined which ligands are responsible for this
process. IL-6 is important in expanding and activating MDSCs43,
and we confirmed that steady-state neutrophils express IL-6Rα,
the IL-6-binding subunit of IL-6R. However, once activated, neutrophils
shed IL-6Rα and most CNS Ly6G+ cells lack IL-6Rα expression in
EAE, consistent with the lack of a clinical phenotype in Il6raΔLy6G-
mice. Nevertheless, because cells lacking IL-6Rα expression can still
receive IL-6 signals through IL-6 trans-signaling or IL-6 trans-
presentation44, the local differentiation of Ly6G+ cells into MDSCs could
occur through cells that have the potential to present IL-6 in
trans.

Because B220+B cells were in close proximity to Ly6G+ cells in the
meningeal space of Stat3ΔLy6G mice, it is intriguing to speculate that

\[ Ly6g^{+} \text{Cre/WT} \quad \text{Stat3}^{+/+} \quad \text{Ly6G}^{+/+} \]

\[ \text{B220} \quad \text{DAPI} \quad \text{Ly6G} \quad \text{pSTAT3} \quad \text{Merge} \]

\[ \text{B220} \quad \text{DAPI} \quad \text{Ly6G} \quad \text{pSTAT3} \quad \text{Merge} \]

\[ \text{B220} \quad \text{DAPI} \quad \text{Ly6G} \quad \text{pSTAT3} \quad \text{Merge} \]

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\[ \text{B220} \quad \text{DAPI} \quad \text{Ly6G} \quad \text{pSTAT3} \quad \text{Merge} \]

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\[ \text{B220} \quad \text{DAPI} \quad \text{Ly6G} \quad \text{pSTAT3} \quad \text{Merge} \]
B cells, which produce IL-6 and can present it in trans through their own IL-6Rα, induce Ly6G+ cell differentiation into MDSCs, which in turn would suppress B cell activation. Consistent with this idea, antigen-stimulated IL-6+ B cells activated STAT3 in Ly6G+ cells only when the cells were in direct physical contact. Their residence in the same anatomical compartment, the meningeal space and superficial CNS parenchyma in the inflamed CNS, might explain the preferential suppression of B cell accumulation by Ly6G+ MDSCs while \(^\text{ex vivo}\) Ly6G+ MDSCs from the CNS express both B cells and T cells\(^{36}\). MOG(35–55)-induced EAE in wild-type C57BL/6 mice is a model of T cell–mediated autoimmunity in the CNS, which is per se independent of B cells\(^{46}\). There are no B cells in the CNS in this model\(^{47}\). B cells might even have a regulatory function in the spleen of MDSCs on germinal center reactions in the CNS needs further investigation.

In conclusion, our study describes an interaction between PMN-MDSCs and a subset of IL-6 and GM-CSF-producing B cells in CNS autoimmunity. These two immune cell subsets are linked in a negative feedback loop. Therapeutic interventions modulating this interaction might prevent the perpetuation of inflammatory responses in the CNS compartment in chronic autoimmune diseases in which local aggregates of B cells are drivers of immunopathology.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at [https://doi.org/10.1038/s41590-018-0237-5].

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Fig. 7 | B cells determine disease progression in the absence of functional MDSCs. a, b. Total numbers of CD19+ B220+ B cells (a) and frequencies of GM-CSF-producing CD19+ B220+ B cells (b) in the CD45+ compartment in the brain and spinal cord of Ly6G+WT and Stat3+/- mice at disease recovery (day 25) when the mice were either control treated (IgG1κ) or i.v. injected with 10 μg-1 CD20 antibody (CD20 Ab) every five days starting on day 12 after immunization; symbols depict mean ± s.d.; Kruskal–Wallis-test with Dunn’s post test; *P < 0.05, **P < 0.01. c, EAE disease course in Ly6G+WT control mice treated with IgG1κ control antibody (n = 4) or CD20 antibody (CD20 Ab, n = 4); symbols depict mean ± s.d.; two-way ANOVA with Bonferroni’s multiple comparison; **P < 0.01. d, EAE disease course in Stat3+/- mice treated with IgG1κ control antibody (n = 5) or CD20 antibody (CD20 Ab, n = 4); symbols depict mean ± s.d.; two-way ANOVA with Bonferroni’s multiple comparison; *P < 0.05.

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Preparation of human PBMCs and flow cytometric analysis of PMBC and CBS cells. PBMCs were isolated using Ficoll density gradient centrifugation (1.077 g ml⁻¹, Merck). Both CSF and PBMC samples were immediately processed after collection. Albumin, IgG, IgM and IgA were measured in cell-free CSF and serum using a BNProSpec (Siemens) analyzer. For cytometry, fresh CSF cells or freshly prepared PBMCs were washed with 2% FCS in PBS, and surface stained with anti-human CD3 (UCHT1), CD4 (R4, T4), CD8 (SK1), CD11b (ICRF44), CD14 (M52), CD15 (H98), CD19 (HIB19), CD33 (P67.6), CD45 (H350, N901), CD138 (M15), HLA-DR (L243) and anti-human LOX1 (15C4), which were purchased from Biologeen, ebioscience or BD Biosciences. Mice. Congenic CD45.1 mice and were obtained from Jackson Laboratory. Ly6G⁻/⁻ mice were generated as described, Strain DBA/2 (Stock No. 55) mice were a gift of F. Getren (Institute for Tumor Biology and Experimental Therapy, Enge-Speyer Haus, Frankfurt, Germany) and Ig50gmc mice were a gift of T. Wunderlich (Max-Planck-Institute for Metabolism Research, Cologne, Germany). To generate mice with cell type–specific excision of loxp-flanked cassettes, mice with loxp-flanked alleles were bred with Ly6G⁻/⁻ mice. All mouse strains were on pure C57BL/6 background. Animals were kept in a specific pathogen-free facility at the University of Essen and the Technical University of Munich. All experimental protocols were approved by the Standing Committee for Experimentation with Laboratory Animals of the administration of Upper Bavaria (AZ 55.2–1–54–232–95–2014, AZ 55.2–1–54–232–29–13, AZ ROB-55.2–2325.Vet.02–17–69, AZ ROB-55.2–2325.Vet.03–18–53) and performed in accordance with the corresponding guidelines.

Induction of EAE, cell depletion and drainage of murine CSF. EAE was induced by subcutaneous application of an emulsion containing 200 μg MOG(35–55) peptide (MEGVYRWSRSPSVHVVLRNGK) and 300 μg Mycobacterium tuberculosis H37Ra (Dnc0) in Freund’s adjuvant oil plus i.v. injection of 200 μg pertussis toxin (Sigma–Aldrich) on days 0 and 2. Mice were rated blindly on a score from 0 to 5 (0 = no disease signs; 1 = tail paresis; 2 = unsteady gait; 3 = paraplegia; 4 = tetraplegia; 5 = death). Mice typically reached disease onset in days 10–12 after immunization, suffered from peak disease in days 13–18 and started to recover in days 20–22 after immunization. Ly6G⁰ neutrophils were depleted by intraperitoneal (i.p.) administration of 400 μg anti-Ly6G (clone 1A8; Bio X Cell). Rat IgG2a served as control (clone 2A3; Bio X Cell). B cells were depleted by i.v. administration of 10 μg pg⁻¹ anti-CD20 every 5 days (clone 18B12, courtesy of the Monoclonal Antibody Core Facility & Research Group, Helmholtz Zentrum Munich, Germany). Mouse IgG1 (clone MOPC-21, Bio X Cell) served as control. For CSF collection, mice were anesthetized with buprenorphine, ketamine and xylazine, and CSF was aspirated from the cisterna magna after burr hole trepanation. Mice underwent an intravascular staining procedure with 50 ng ml⁻¹ anti-IL-6R (Sigma–Aldrich) and 100 ng ml⁻¹ IL-4 (Miltenyi Biotec) at 37 °C and 5% CO₂ for 2 h and were cocultured with Ly6G⁰ suppressor cells in different ratios. To test blocking of suppression, 0.5 μM N-Monomethyl-l-arginine (l-NMA, NOS2 inhibitor, Sigma–Aldrich), 0.5 μM N-Hydroxy-l-arginine (l-NOHA, Arginase 1 inhibitor, Sigma–Aldrich), 5 μM anti-CD27 (anti-PD-1, 10E6G2, Biologeen), or 5 μM anti-VISTA (MH5A, Biologeen) were added. For human B cell proliferation assays, responder CD19⁺ B2B⁺ B cells were highly purified by FACs sorting from naïve peripheral blood (PB) of healthy donors. 2,000 or 5,000 B cells were stimulated in culture medium containing 5% FCS with 50 μg pg⁻¹ anti-CD40 (FGK4.5, Bio X Cell) and 10 ng ml⁻¹ IL-4 (Miltenyi Biotec) at 37 °C and 5% CO₂ for 72 h and were cocultured with Ly6G⁰ suppressor cells in different ratios. To test blocking of suppression, 0.5 μM N-Monomethyl-l-arginine (l-NMA, NOS2 inhibitor, Sigma–Aldrich), 0.5 μM N-Hydroxy-l-arginine (l-NOHA, Arginase 1 inhibitor, Sigma–Aldrich), 5 μM anti-CD27 (anti-PD-1, 10E6G2, Biologeen), or 5 μM anti-VISTA (MH5A, Biologeen) were added. For human B cell proliferation assays, responder CD19⁺ B2B⁺ responder B cells and CD19⁺ CD11b⁺ CD33⁺ LOX1⁺ MDCs were purified from PBMC samples of the same individual and plated in U-bottom plates. CD19⁺ CD11b⁺ CD33⁺ LOX1⁺ neutrophils were purified from whole-blood samples of the same individuals after erythrocyte lysis served as controls. In each well, 8,000 B cells were stimulated with 1 μg pg⁻¹ anti-CD40 (G28.25, Bio X Cell) and 20 μg pg⁻¹ IL-4 (Miltenyi Biotec) at 37 °C and 5% CO₂ for 72 h and were cocultured with MDCs or neutrophils in different ratios. After 72 h, all wells were pulsed with 1 μCi [³H]thymidine and harvested 19 h later for assay of thymidine incorporation by scintillation counting (PerkinElmer). All conditions were run as triplicates.

Transwell in vitro culture. TCR transgenic MOG-specific CD4⁺ T cells and BCR transgenic MOG–specific CD19⁺ B cells were FACS-purified from naive 2D mice and “th” mice, respectively. Some 100,000 T and 100,000 B cells per well were co-cultured with recombinant rat MOG(1–125) (Biotrend) at 48 h. Splenic Ly6G⁻ CD11b⁺ cells were FACS-purified from wild-type mice on day 7 after immunization with CFA, and 125,000 Ly6G⁻ CD11b⁺ cells were then added to the T cell-B cell coculture into the bottom compartment of a transwell system (HTS Transwell 96-well plate, 0.4 μm polycarbonate membrane, Corning, Sigma–Aldrich) or physically separated from the T-B coculture in the top compartment in the presence of 2 μg pg⁻¹ anti-IL-6R (MR1–16) or 25 μg pg⁻¹ rat IgG2a (clone 2A3; Bio X Cell). Additionally, 125,000 Ly6G⁻ CD11b⁺ cells alone were either stimulated with 50 μg pg⁻¹ IL-6 (Miltenyi Biotec) or equimolar amounts of human IL-6-IL-6R (a gift of Stefan Rose-John, Kiel, Germany) in the presence of 25 μg pg⁻¹ anti-IL-6R or 25 μg pg⁻¹ rat IgG2a. After 20 h, cells were analyzed for pSTAT3 using the BD Phosflow Lyse/Fix Buffer and BD Phosflow Perm Buffer III (BD Biosciences) according to the manufacturer’s instructions and stained with fluorochrome-conjugated anti-mouse TRSTAX p70S (4/4-STAT3), CD4 (RM4–5), CD11b (M1/70), CD19 (D3) and Ly6G (1A8). Flow cytometric analysis was performed on a CytoFLEX flow cytometer (Beckman Coulter) and flow cytometric data were analyzed using FlowJo software (Tree Star). FACs sorting was performed on a FACs Aria III (BD Biosciences).

Analysis of cytokine and chemokine levels in murine CSF. Fresh CSF samples were cautiously thawed. Cytokine and chemokine levels were measured in 5 μl CSF using a bead-based immunoassay according to the manufacturer’s instructions (LEGENDEXplex, Biologeen). Analysis was down using a CytoFLEX flow cytometer (Beckman Coulter).

B cell proliferation suppression assay. For murine B cell proliferation assays, responder CD19⁺ B2B⁺ B cells were highly purified by FACs sorting from naïve spleen of healthy donors. 96-well U-bottom plates were seeded with 2,000 or 5,000 B cells were stimulated in culture medium containing 5% FCS with 50 μg pg⁻¹ anti-CD40 (FGK4.5, Bio X Cell) and 10 ng ml⁻¹ IL-4 (Miltenyi Biotec) at 37 °C and 5% CO₂ for 72 h and were cocultured with Ly6G⁰ suppressor cells in different ratios. To test blocking of suppression, 0.5 μM N-Monomethyl-l-arginine (l-NMA, NOS2 inhibitor, Sigma–Aldrich), 0.5 μM N-Hydroxy-l-arginine (l-NOHA, Arginase 1 inhibitor, Sigma–Aldrich), 5 μg pg⁻¹ anti-CD27 (anti-PD-1, 10E6G2, Biologeen), or 5 μg pg⁻¹ anti-VISTA (MH5A, Biologeen) were added. For human B cell proliferation assays, responder CD19⁺ CD11b⁺ CD33⁺ LOX1⁺ MDCs were purified from PBMC samples of the same individual and plated in U-bottom plates. CD19⁺ CD11b⁺ CD33⁺ LOX1⁺ neutrophils were purified from whole-blood samples of the same individuals. After erythrocyte lysis served as controls. In each well, 8,000 B cells were stimulated with 1 μg pg⁻¹ anti-CD40 (G28.25, Bio X Cell) and 20 μg pg⁻¹ IL-4 (Miltenyi Biotec) at 37 °C and 5% CO₂ for 72 h and were cocultured with MDCs or neutrophils in different ratios. After 72 h, all wells were pulsed with 1 μCi [³H]thymidine and harvested 19 h later for assay of thymidine incorporation by scintillation counting (PerkinElmer). All conditions were run as triplicates.
with citrate buffer (pH 6). Endogenous peroxidases (peroxidase blocking reagent, Dako) were neutralized and nonspecific binding was blocked for 5 min (PBS/1% BSA/2% FCS). For brightfield microscopy, tissue sections were incubated with rat anti-mouse B220 (clone RA3-6B2). Bound primary antibodies were visualized with biotin-labeled anti-rabbit antibody and streptavidin-peroxidase staining method using polymerized 3,3′-diaminobenzidine (all reagents from Dako; hemaluna counterstaining of nuclei). For immunofluorescence, sections were blocked for 5 min (PBS/1% BSA/2% FCS) and subsequently incubated with rat anti-mouse Ly6G (clone 1A8). Bound primary antibodies were visualized by using goat anti-rabbit horseradish peroxidase and tyramide signal amplification (Alexa488, Life Technologies). Subsequently, sections were incubated with rabbit anti-pSTAT3 (clone D3A7, Cell Signaling Technology) and bound antibodies were visualized with Alexa555-labeled goat anti-rabbit antibody. Finally, slides were incubated with 647-labeled rat anti-B220 (clone RA3-6B2) and nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, Invitrogen). For further stainings, rat anti-mouse CD19 (clone 60MP31, eBioscience) and polyclonal rabbit anti-mouse CD3 (Dako) were used. Immunostained sections were scanned using Pannoramic Digital Slide Scanner 250 FLASH II (3DHistech) in ×200 magnification. For cytology, cells were highly purified ex vivo by FACS sorting. After Cytospin centrifugation (Thermo Fisher), May–Gruenwald–Giems staining was performed according to the manufacturer’s instructions (Merck).

Quantitative PCR. Total RNA was isolated with RNeasy Plus micro kit (Qiagen). The isolated RNA was transcribed into cDNA using the TaqMan Reverse Transcription Reagents Kit (Life Technologies) according to the manufacturer’s instructions. Probes were purchased from Life Technologies and the assays were performed in 96-well reaction plates (Life Technologies). Real-time PCR was performed on a StepOnePlus system (Life Technologies). In all experiments Actb was used as a reference gene to normalize gene expression. All samples were run as triplicates.

RNA sequencing. Total RNA was isolated with RNeasy Plus mini kit (Qiagen). Quality and integrity of total RNA was controlled on an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Santa Clara). For RNA-seq analysis, library preparation for bulk 3′ sequencing of poly(A)-RNA was done as described. Briefly, barcoded cDNA of each sample was generated with a Maxima RT polymerase (Thermo Fisher) using oligo-dT primer containing barcodes, unique molecular identifiers (UMIs) and an adapter. The 5′ ends of the cDNAs were extended by a template switch oligonucleotide and full-length cDNA was amplified with primers binding to the template switch oligonucleotide site and the adapter. cDNA was taggedmented with the Nextera XT kit (Illumina) and 3′-end fragments were finally amplified using primers with Illumina P5 and P7 overhangs. In comparison to the work of Parekh et al., the P5 and P7 sites were exchanged to allow sequencing of the cDNA in read1 and barcodes and UMIs in read2 to achieve better cluster recognition. The library was sequenced on a NextSeq 500 (Illumina) with 75 cycles for the cDNA in read1, and 16 cycles for the barcodes and UMIs in read2. Data were processed using the published Drop-seq pipeline (version 1.0) to generate sample- and gene-wise UMI tables. A reference genome (GRCm38) was used for alignment. Transcript and gene definitions were used according to ENSEMBL annotation release 91. Differential gene expression analysis was conducted with DESeq version 1.18.1 (ref. 15). After fitting a model with all samples, contrasts of interest were extracted. Expression differences between CNS onset and the remaining samples were determined. Then contrasts between all possible pairwise combinations of the other groups, except the CNS-onset samples, were extracted. Genes exclusively regulated in the CNS-onset group were subjected to pathway analysis with GOrellia. We considered genes with a false discovery rate (FDR) ≤ 0.05 and an at least two-fold absolute expression difference between groups to be differentially expressed. For principal component analysis the top 2,000 variable genes were used as input.

Statistics. Statistical evaluations of cell frequency measurements, cell numbers, mRNA amounts, chemokine levels and enzyme activity were performed with the unpaired Student’s t-test or Mann–Whitney U-test, as indicated in figure legends, when two populations were compared. Two-tailed P values < 0.05 were considered significant. Multiple comparisons were performed with one-way analysis of variance (ANOVA) and post-hoc multiple comparison tests as indicated in figure legends. EAE scores between groups were analyzed as disease burden per individual day with two-way ANOVA and Bonferroni post-testing. P < 0.05 was considered significant. If not otherwise stated, values are given as mean ± s.d. Calculations were performed using Graph Pad Prism v7.04 (Graph-Pad software) or R software (R Core Team 2015, version 3.5.1).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability RNA-seq data have been deposited in the European Nucleotide Archive under accession code PRJEB28339. All data that support the findings of this study are available from the corresponding author upon reasonable request.

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Policy information about availability of computer code

Data collection
- BD FACS Diva Software v8.0.1; CytExpert v2.1.0.92; StepOne Software v2.3; MicroBeta Windows workstation v4.70.05

Data analysis
- Graph Pad Prism v7.04 (Graph-Pad software, La Jolla, USA); FlowJo v10.4.2 (FlowJo, LLC, Oregon, USA) GOriilla (https://doi.org/10.1186/1471-2105-10-48) R software (R Core Team 2015, version 3.5.1)

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Raw data in Figures (Fig. 3A, B, C; 5B; suppl. Fig 2) will be provided. The accession code of our RNA-seq data with GEO is PRJEB28339.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
- Murine experiments: No a-priori sample size calculation was performed since there were no preliminary data with the experimental systems that were used. Thus, our study was exploratory. We always used a minimum of 6 animals per group. This is based on the observation that minimum and maximum measure in a group of n=6 corresponds to the 95% confidence interval for the median.
- Human experiments: No a-priori sample size calculation was performed. Again, this was an exploratory study and needs to be validated with the now existing preliminary data in larger cohorts.

**Data exclusions**
- The complete dataset on any individual human or murine sample was excluded if any obvious problem during sample processing occurred, i.e. more than 70 percent dead cells on live dead staining.

**Replication**
- The majority of all experiments were repeated for reproducibility as stated in the respective figure legends. All replications worked.

**Randomization**
- Murine experiments: Only mice bred on pure C57BL/6 background were used for the experiments. We used mice aged from 10-14 weeks, whereas in all experiments different groups were both sex- and age-matched and all experiments were performed within the same mouse SPF facility. Due to the homogenous genetic background, there was no need to control for further covariates.
- Human experiments: As described in the figure legends, statistical analysis was corrected for the covariate age.

**Blinding**
- Murine experiments: EAE disease courses were rated in a blinded manner. Further analysis was performed unblinded. Every experiment was performed using several control samples and the applied analysis strategy (especially FACS gating strategy) was identical for every single sample.
- Human experiments: During analysis, patient data underwent pseudonymisation and was blinded to the analyser.

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| Involved in the study          | Involved in the study |
| Unique biological materials    | ChIP-seq |
| Antibodies                     | Flow cytometry |
| Eukaryotic cell lines          | MRI-based neuroimaging |
| Palaeontology                  |         |
| Animals and other organisms    |         |
| Human research participants    |         |

**Antibodies**

Antibodies used:
- Flow cytometry:
  - anti-human CD3 (UCHT1), APC-Cy7, BD Bioscience, 341110
  - anti-human CD4 (RPA_T4), PerCP, BD Bioscience, 345770
  - anti-human CD8 (SK1), PE-Cy7, Beckman Coulter, 737661
  - anti-human CD11b (ICRF44), PE-Cy7, BD Bioscience, 557743
  - anti-human CD14 (MSE2), APC, BD Bioscience, 553899
  - anti-human CD15 (HI98), FITC, BD Bioscience, 555401
  - anti-human CD19 (HIB19), ECD, Beckman Coulter, A07770
  - anti-human CD33 (P67.6), BV421, BD Bioscience, 562854
  - anti-human CD45 (HI30), BV605, BD Bioscience, 564047
  - anti-human CD56 (N901), APC, Beckman Coulter, IM2474
  - anti-human CD138 (MI15), PE, Beckman Coulter, AS4190
anti-human HLA-DR (L243), PE, Biolegend, 307628
anti-human LOX1 (15C4), PE, Biolegend, 358604

anti-mouse CD1d (1B1), PE, BD, 553846
anti-mouse CD3 (145-2C11), FITC, eBioscience, 11-0031
anti-mouse CD3 (145-2C11), PB, BD Bioscience, 558214
anti-mouse CD4 (RM4-5), APC, BD Bioscience, 553051
anti-mouse CD4 (RM4-5), APC-ef780, eBioscience, 47-0042
anti-mouse CDS (53-7.3), APC-R700, BD, 565505
anti-mouse CD11b (M1/70), PerCP-Cy5.5, BD Bioscience, 550993
anti-mouse CD19 (1D3), PE-Cy7, eBioscience, 25-0193
anti-mouse CD19 (1D3), BV510, Biolegend, 115546
anti-mouse CD19 (1D3), BV605, Biolegend, 504508
anti-mouse CD19 (6D5), BV785, Biolegend, 115543
anti-mouse CD21/CD35 (7E9), FITC, Biolegend, 123407
anti-mouse CD23 (B384), BV421, BD Bioscience, 562929
anti-mouse CD24 (M1/69), PerCP-Cy5.5, Biolegend, 103212
anti-mouse CD24 (M1/69), BV510, Biolegend, 103212
anti-mouse CD25 (53-7.3), APC-R700, BD, 565532
anti-mouse CD45R/B220 (RA3-6B2), purified, eBioscience, 14-0452
anti-mouse CD45R/B220 (RA3-6B2), purified, BD Bioscience, 551459
anti-mouse CD45R/B220 (RA3-6B2), purified, Biolegend, 103227

Several lots were used of all antibodies, which did not affect in any way the results. With all lots the results were reproducible.

Validation

All primary antibodies are commercially available antibodies, all antibodies underwent quality control by the manufacturer and are applicable to flow cytometry or histology (as stated above)

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals
- Congenic CD45.1 mice: Jackson Laboratory (B6 Cd45.1, Stock No: 002014);
- Il6stflox/flox (Il6sttm1Wme): Jackson Laboratory (B6N(Cg)-Il6sttm1b(KOMP)Mbp/J; Stock No: 022388);
- Ly6gCre/WT mice: Generated as previously described (DOI: 10.1038/nmeth.3322);
- Cd45.1 (A20), purified, eBioscience, 127633
- Cd19 (60MP31), purified, eBioscience, 14-0194-80
- STAT3 pY705 (D3A7), purified, Cell Signaling Technology, 9145S
- Anti-mouse CD11b (M1/70), purified, Dako, A0452
- Goat anti-rabbit IgG secondary antibody, Alexa488, Life technologies, A-11008
- Goat anti-rabbit IgG secondary antibody, Alexa555, Life technologies, A-21428

Histology:

Goat anti-rabbit IgG secondary antibody, Alexa488, Life technologies, A-11008
Goat anti-rabbit IgG secondary antibody, Alexa555, Life technologies, A-21428

Several lots were used of all antibodies, which did not affect in any way the results. With all lots the results were reproducible.
All experimental mice were used at the age of 10 to 14 weeks.

Wild animals
No wild animals were used.

Field-collected samples
No field-collected samples were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics
In total, 70 patients with clinically isolated syndrome (n=32) or relapsing remitting multiple sclerosis (n=38) were included into the study and underwent analysis of peripheral blood mononuclear cells (PBMCs). 31 healthy individuals served as controls. Population characteristics are provided in the supplementary figure 1 b.

25 patients with CIS or RRMS underwent analysis of the cerebrospinal fluid (CSF), population characteristics are also provided within the supplementary Figure 1 b. The most relevant co-variate for LOX1+ PMN-MDSCs is age. All comparisons were corrected for age as a co-variate.

10 patients with CIS or RRMS underwent a prospective follow-up over up to 12 months. Patient characteristics are provided in the supplementary Figure 1 c.

Recruitment
Patients with diagnosis of relapsing remitting MS or clinically isolated syndrome (CIS) between 18 and 60 years of age were recruited from our department between 2017 and 2018. Exclusion criteria were any non-MS-specific immunomodulatory treatments, any steroid treatment within the last 14 days and any hematologic diseases.

Flow Cytometry

Plots
Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Human blood and CSF samples:
PBMCs were isolated using Ficoll density gradient centrifugation (1.077 g/ml, Merck, Darmstadt, Germany). Both CSF and PBMC samples were immediately processed after collection. Fresh CSF cells or freshly prepared PBMCs were washed with 2% fetal calf serum/phosphate-buffered saline and surface stained using anti-human antibodies as described for 20 minutes (room temperature).

Murine samples:
Mice were anesthetized with isoflurane or with buprenorphine, ketamine and xylazine. After perfusion through the left cardiac ventricle with cold PBS, brain and spleen were dissected; the spinal cord was flushed out of the spinal canal with PBS by hydrostatic pressure. The spinal cord was dissected from the brain at the level of the medulla oblongata, optic nerves were dissected stereo-microscopically between the optic disc and the optic chiasm. CNS tissues were digested separately with 1.0 mg/ml collagenase d (Roche, Basel, Switzerland) and 40 μg/ml DNaseI (Sigma-Aldrich) at 37°C for 45 minutes. CNS cells were isolated by passing the tissue through a 70 μm cell strainer and by 37% Percoll gradient centrifugation. Splenic tissue was passed through a 70 μm cell strainer, erythrocyte lysis was performed afterwards. The cell pellet was washed and resuspended in culture medium for further analysis. For surface stainings, cell suspensions were treated with Fc block CD16/CD32 (2.4G2) and surface markers were stained with fluorochrome conjugated anti-mouse antibody. For staining of intracellular molecules (not cytokines), cells were fixated and premeabilized using Foxp3 staining kit (eBioscience, Thermo Fisher, Waltham, USA). For intracellular cytokine staining, cells were stimulated in culture medium containing phorbol 12-myristate 13-acetate (PMA, 20 ng/ml, SigmaAldrich), ionomycin (1 microg/ml, Sigma-Aldrich), and monensin (GolgiStop 1 microl/ml, BD Biosciences) at 37 degreeC/5% CO2 for 2 hours. Afterwards, Foxp3 staining kit (eBioscience, Thermo Fisher, Waltham, USA) was used for fixation and permeabilization of cells and cells were stained with the respective primary antibodies at 4 degree C over night.

Instrument
Beckman Coulter, Cyan ADP 7 colour, Model No not available.
Beckman Coulter, Cytoflex flow cytometer, Cytoflex S, Model No B75442 BD,
FACS ARIA III, Model No P648050C1002
## Software
FlowJo software, Tree Star, Ashland, USA, v10

## Cell population abundance

All samples were sorted using "high-purity" mode and every sample underwent a post-sort purity check. If sort purity was < 90.0% of the FSC/SSC gate of starting cell population, the sample was excluded and not used for further experiments.

Cell population abundance in sorted samples:

| Cell Population | Percentage |
|-----------------|------------|
| Ly6G+ neutrophils (donor naive spleen) | 5-10% |
| Ly6G+ neutrophils (donor immunized spleen) | 50-70% |
| Ly6G+ neutrophils (donor CNS at EAE recovery) | 3-8% |
| CD19+B220+ B cells (donor naive spleen) | 40-60% |

## Gating strategy

FSC-A/SSC-A -> FSC-H/FSC-W -> SSC-H/SSC-W -> FSC-A/Live-Dead -> cells of interest defined as "alive cells". See supplementary Figure 4 d.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.