Hypoxia-inducible factor-1α is a critical transcription factor for IL-10-producing B cells in autoimmune disease

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Hypoxia-inducible factors (HIFs) are key elements for controlling immune cell metabolism and functions. While HIFs are known to be involved in T cells and macrophages activation, their functions in B lymphocytes are poorly defined. Here, we show that hypoxia-inducible factor-1α (HIF-1α) contributes to IL-10 production by B cells. HIF-1α regulates IL-10 expression, and HIF-1α-dependent glycolysis facilitates CD1dhiCD5+ B cells expansion. Mice with B cell-specific deletion of Hif1a have reduced number of IL-10-producing B cells, which result in exacerbated collagen-induced arthritis and experimental autoimmune encephalomyelitis. Wild-type CD1dhiCD5+ B cells, but not Hif1a-deficient CD1dhiCD5+ B cells, protect recipient mice from autoimmune disease, while the protective function of Hif1a-deficient CD1dhiCD5+ B cells is restored when their defective IL-10 expression is genetically corrected. Taken together, this study demonstrates the key function of the hypoxia-associated transcription factor HIF-1α in driving IL-10 expression in CD1dhiCD5+ B cells, and in controlling their protective activity in autoimmune disease.

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**Results**

**HIF-1α expression increases in activated B cells.** To investigate the role of HIFs in B cells, the expression of HIF-1α and HIF-2α was determined in C57BL/6 WT splenic B cells stimulated with lipopolysaccharide (LPS) (10 μg/ml) or anti-IgM (10 μg/ml). Even under normoxic conditions, Hif1a mRNA expression is induced in B cells stimulated with LPS or anti-IgM (Fig. 2a), whereas the expression of Hif2a is almost undetectable and remains unchanged when analyzed in fold change (Fig. 2a). Accordingly, HIF-2α protein is hardly detectable, whereas HIF-1α protein increases at 4, 8, and 12 h after LPS or anti-IgM stimulation in B cells (Fig. 1b). Since HIF-1α induction by LPS has been already reported to be dependent on NF-κB signaling, we also checked whether this pathway is effective in B cells. Indeed, knockdown of RelA not only decreases p65 phosphorylation but also HIF-1α protein level in B cells stimulated by LPS for 4 h (Supplementary Fig. 1a).

Since B cells stimulation by anti-IgM also induces HIF-1α (Fig. 1b), we delineated the pathways of HIF-1α induction in BCR-stimulated B cells. Therefore, ERK and STAT3 proteins levels were analyzed. As shown in Fig. 1c, phosphorylated-ERK (pERK) and phosphorylated-STAT3 Ser727 (pSTAT3Ser727) are increased in splenic B cells after anti-IgM stimulation, whereas phosphorylated-STAT3 Tyr705 (pSTAT3Tyr705) is virtually undetectable. Using specific inhibitor of ERK, STAT3, and AKT pathways, which are not affecting B cell viability (Supplementary Fig. 1b), we analyzed the pathway essential for HIF-1α protein expression. Indeed, HIF-1α protein induction is suppressed in a dose-dependent manner when BCR-stimulated B cells are treated with ERK or STAT3 inhibitors, but not AKT inhibitor treatment (Fig. 1d). Similarly, decrease of HIF-1α protein is observed when STAT3 or ERK are knocked down in B cells using siRNA approach (Supplementary Fig. 1c). Interestingly, STAT3Ser727 phosphorylation is decreased after B cell treatment with ERK inhibitor (Fig. 1d), suggesting that phosphorylation of ERK is essential for STAT3Ser727 phosphorylation.

Next, we determined whether pSTAT3Ser727 could also transcriptionally regulate Hif1a gene expression. To do so, chromatin immunoprecipitation (ChIP) analysis was performed on a putative STAT3 binding site on Hif1a promoter at ~309 bp/319 bp from the transcription starting site (TSS) (Fig. 1e). Indeed, low level of pSTAT3Ser727 can bind to Hif1a promoter in splenic B cells in homeostasis (Fig. 1e). Interestingly, pSTAT3Ser727 binding on Hif1a promoter is strikingly enhanced in BCR-mediated activated B cells (Fig. 1e). Our results demonstrate that HIF-1α is increased in mRNA and protein levels in LPS-treated B cells via the NF-κB pathway and in BCR-stimulated B cells via ERK–STAT3 activation.

**B1a population is reduced in Mb1creHif1af/f mice.** To determine the roles of HIF-1α and HIF-2α during B cell development in vivo, we bred mice carrying a loxP-flanked Hif1a or Hif2a allele with mice expressing cre recombinase from the Mb1 promoter to delete Hif1a or Hif2a specifically in B lymphocytes (referred to herein as Mb1creHif1af/f or Mb1creHif2af/f mice). As expected, HIF-1α or HIF-2α protein is completely abolished in splenic B cells but not in T cells isolated from Mb1creHif1af/f or Mb1creHif2af/f mice compared with WT control mice (Supplementary Fig. 1d). Next, flow cytometric analysis of the B cell subpopulations in Mb1creHif1af/f or Mb1creHif2af/f, and WT control mice were performed (Fig. 2a). No difference can be detected in the populations of pre-pro-B, pro-B, pre-B, immature, and recirculating B cells (Hardy fractions A–F) in WT and mutant mice (Fig. 2b). The splenic B cell subpopulations, transitional type 1 and 2 as well as follicular cells, are also similar in Mb1creHif1af/f, Mb1creHif2af/f, and control mice (Fig. 2c, d). However, percentage and absolute numbers of marginal zone B cells are moderately decreased in Mb1creHif1af/f mice compared to WT mice (Fig. 2d).

Next, we analyzed peripheral B cell subsets in inguinal lymph nodes and blood from Mb1creHif1af/f, Mb1creHif2af/f, and WT mice. HIF-1α or HIF-2α deletion does not alter the immature and mature B cell populations in the periphery (Fig. 2e, f). Interestingly, only B1a cell number is drastically decreased in the peritoneum of Mb1creHif1af/f mice when compared to WT or Mb1creHif2af/f mice, whereas no difference is observed for B1b cells (Fig. 2g).
To further determine the effects of HIF-1α and HIF-2α on B cell functions in vitro, proliferation and apoptosis rates were examined in splenic B cells stimulated with lipopolysaccharide (LPS) or anti-IgM at indicated time points ($n = 4$ at each time point). Values at 0 h were set as 1. b Expression of HIF-1α and HIF-2α in WT splenic B cells stimulated with LPS or anti-IgM at indicated time points ($n = 3$ experiments in duplicate). c Western blot and densitometry analysis of phospho-STAT3 Ser727 (pSTAT3$^{727}$), phospho-STAT3 Tyr705 (pSTAT3$^{705}$), total STAT3, phospho-ERK (pERK), and total ERK in WT splenic B cells after stimulation with anti-IgM at indicated time points ($n = 3$ experiments in duplicate). d Western blot and densitometry analysis of HIF-1α, pSTAT3$^{727}$, and pERK in anti-IgM-stimulated B cells with or without STAT3, ERK, or AKT inhibitors treatments for 4 h ($n = 3$ experiments in duplicate). e Scheme of Hif1a promoter indicating the potential STAT3 binding site position and enrichment of pSTAT3$^{727}$ on Hif1a promoter in splenic B cells 4 h after stimulation with anti-IgM or medium (Med) ($n = 4$ for all groups). Data are shown as mean ± s.e.m. Pictures are representative of three (a-d) or four (e) independent experiments. *$P < 0.05$; **$P < 0.01$, and ***$P < 0.001$ (two-tailed unpaired Student’s t-test) (see also Supplementary Figure 1)
HIF-1α deficiency causes CD1dhiCD5+ B cell defects. Previous studies have shown that B1a cells possess regulatory functions and produce the anti-inflammatory cytokine IL-10 after activation. To address whether IL-10 is altered by the loss of HIF-1α or HIF-2α in B cells, IL-10 intracellular staining in B cells was performed. As shown in Fig. 3a, the frequency of IL-10 positive (IL-10+) B cells is decreased in bone marrow, spleen, inguinal lymph nodes, and peritoneal cavity of Mb1creHif1af/f mice compared to Mb1creHif2af/f or WT mice. In accordance, HIF-1α intracellular staining in IL-10+ and IL-10− B cells reveal an
increased level of HIF-1α protein in IL-10− B cells (Supplementary Fig. 3a). Because IL-10-producing B cells have been described in different B cell subpopulations such as CD1dhiCD5+CD19− B cells22 and CD23−CD21+IgM−(T2-MZP) B cells23, we speculated that these two subsets are modified in Mb1creHif1af/f mice in vivo. It is noteworthy that percentage and absolute numbers of CD1dhiCD5+ or T2-MZP B cells are reduced in Mb1creHif1af/f mice compared to WT littermates (Fig. 3b and Supplementary Fig. 3b). Less BrdU-positive cells are observed in CD1dhiCD5+ and T2-MZP populations of Mb1creHif1af/f mice compared to WT control mice (Fig. 3c and Supplementary Fig. 3c), implying a defect of regulatory B cell proliferation in Hif1α-deficient mice. In accordance, IL-10 mRNA expression is significantly increased in Mb1creHif1af/f mice (Fig. 3d). Moreover, analysis of anti-inflammatory cytokines expression in sorted CD1dhiCD5+ B cells from Mb1creHif1af/f mice reveals a significant decrease in Il10 mRNA expression as well as a decreased IL-10 production after stimulation (*P < 0.05 and **P < 0.01, by t-test; Fig. 3e and Supplementary Fig. 3d), whereas Tgfb, P35, and Ebi3 mRNA levels are not altered (Supplementary Fig. 3d). Altogether, these data suggest that HIF-1α is an important factor for the expansion of CD1dhiCD5+ B cells, and their IL-10 production.

**HIF-1α regulates glycolysis in CD1dhiCD5+ B cells.** Since CD1dhiCD5+ B cell number is normal in Il10-deficient mice24, we hypothesized that the reduced IL-10 level is likely not responsible for the reduced CD1dhiCD5+ B cell number in Mb1creHif1af/f mice. HIF-1α was previously identified as a key factor for glycolytic activity and glucose metabolism in immune cell function and proliferation22–27. To further dissect the expansion of CD1dhiCD5+ B cells, we examined the level of HIF-1α in this population. Indeed, HIF-1α protein level is higher in CD1dhiCD5+ B cells than in CD1dloCD5− B cells (Supplementary Fig. 4a). Next, glucose uptake was examined in FACSorted CD1dhiCD5+ and CD1dloCD5− B cells from WT mice. CD1dhiCD5+ B cells display a two-fold increase in glucose transport activity compared to CD1dloCD5− B cells (Fig. 4a), suggesting that CD1dhiCD5+ B cells preferentially use glucose metabolism. Moreover, CD1dhiCD5+ B cells from Mb1creHif1af/f mice exhibit a lower level of glucose uptake and lactate secretion (Fig. 4b, c) compared to CD1dhiCD5+ B cells from WT mice, whereas no difference in glucose uptake between Hif1α-deficient and WT CD1dhiCD5+ B cells (Supplementary Fig. 4b). Accordingly, mRNAs expression of HIF-1α-targeted glycolytic genes, glucose transporter 1 (Glut1), pyruvate kinase M2 (Pkm2), hexokinase 2 (Hk2), lactate dehydrogenase A (LdhA), phosphoinositide-dependent kinase 1 (Pdk1), and glucose-6-phosphate isomerase 1 (Gpi1), are markedly decreased in Hif1α-deficient CD1dhiCD5+ B cells compared to WT CD1dhiCD5+ B cells (Fig. 4d). Next, we delineated whether the high glycolytic activity of CD1dhiCD5+ B cells was critical for the expansion of CD1dhiCD5+ B cells. As shown in Fig. 4e, partial inhibition of glycolysis by treatment with competitive glycolytic inhibitor 2-deoxyglucose is sufficient to inhibit WT CD1dhiCD5+ B cells proliferation to a similar level as found in untreated Hif1α-deficient CD1dhiCD5+ B cells. Taken together, these data suggest that HIF-1α expression controls the expansion of CD1dhiCD5+ B cells by orchestrating their high glycolytic activity.

**HIF-1α and STAT3 cooperatively regulate Il10 transcription.** To delineate how HIF-1α can regulate IL-10 expression in B cells, splenic B cells from Mb1creHif1af/f and WT mice were cultured under normoxic or hypoxic condition. Interestingly, Il10 mRNA expression is strongly increased in B cells cultured under hypoxia compared to normoxia (Fig. 5a). Consistent with the reduced IL-10 production in Hif1α-deficient B cells (Fig. 3), Il10 mRNA expression is also lower in Hif1α-deficient B cells than WT B cells under hypoxic condition (Fig. 5a). We next examined whether Il10 gene expression could be transcriptionally regulated by HIFs in B cells. Bio-informatics promoter analysis using JASPA with the consensus core (A/GCCGTG), reveals several putative hypoxia-responsive element (HRE) regions (I–V) on Il10 promoter (Fig. 5b and Supplementary Fig. 5a). By ChIP assay, we show that HIF-1α can bind to HRE I and HRE II regions under hypoxic condition (Fig. 5c). Interestingly, the pattern of HIF-1α binding is similar to that of histone H3 (trimethylK4) antibodies, whereas no specific binding is detected when using control IgG antibodies (Fig. 5d and Supplementary Fig. 5b), suggesting that these regions are transcriptionally active under hypoxia. Next, luciferase reporter assays with putative HRE constructs were performed in 293T cells after hypoxic or normoxic culture. As expected, the luciferase activity of the HRE I and HRE II constructs are increased under hypoxic condition, suggesting that HIF-1α activates Il10 transcription through HRE I and HRE II regions (Fig. 5e).

Since STAT3 and HIF-1α were previously shown to cooperate on HIF target genes such as Ca9 and Pkg128, we hypothesized that the highly expressed pSTAT3327 in BCR-activated B cells (Fig. 1c) might form a complex with HIF-1α to activate Il10 transcription. To test this hypothesis, we confirmed the binding of HIF-1α in B cells after anti-IgM stimulation (Fig. 5f). In addition, HIF-1β can also bind to the HRE I and HRE II regions on the Il10 promoter in B cell after anti-IgM stimulation, whereas no binding of HIF-2α or control IgG is detected (Supplementary Fig. 5g).

**Fig. 2** B1a cell number is reduced in the peritoneal cavity of Mb1creHif1af/f mice. a Scheme of developmental, maturation, and migration stages of B cells in bone marrow, spleen, peritoneal cavity, lymph node, and blood. Arrows indicate most likely developmental pathway and dotted arrows indicate still debated pathway. b Representative plots and absolute numbers of B-cell subpopulations in bone marrow from Mb1creHif1af/f (n = 8), Mb1creHif2af/f (n = 8), and WT control (n = 8) (cre-negative floxed) mice. The subpopulations were separated into six populations (fractions A–F) according to the Hardy classification, A: pre-pro-B (B220−CD43−BP-1−CD24−); B: pro-B (B220−CD43−BP-1−CD24+); C: late pre-B (B220−CD43−BP-1−CD24med); D: early pre-B (B220−CD43−BP-1−CD24−); E: late pre-B (B220−CD43+IgD−IgM+); F: immature B (B220−CD43+IgD−IgM+); and: F: recirculating B (B220−CD43+IgD−IgM+). c Representative plots and absolute numbers of transitional type 1 and transitional type 2 B cells in spleen from Mb1creHif1af/f (n = 8), Mb1creHif2af/f (n = 8), and WT mice (n = 8). T1: transitional type 1 B cells (B220+AA4.1+IgM+CD23−); T2: transitional type 2 B cells (B220+AA4.1+IgM+CD23+). d Representative plots and absolute numbers of follicular B cells and marginal zone B cells in spleen from Mb1creHif1af/f (n = 10), Mb1creHif2af/f (n = 10), and WT mice (n = 10). FO: follicular B cells (B220+AA4.1+CD23−CD21+); Mz: marginal zone B cells (B220+AA4.1+CD23+CD21+IgM−IgD−CD19+). e, f Representative plots and absolute numbers of B-cell subpopulations in lymph node (e) or blood (f) from Mb1creHif1af/f (n = 8), Mb1creHif2af/f (n = 8), and WT mice (n = 8). M: mature B (B220+CD19−IgD−IgM−); IM: immature B (B220+CD19−IgD−IgM+). g Representative plots and absolute numbers of B1a (CD19−IgM+CD11b−CD5−) and B1b (CD19+IgM+CD11b−CD5+) cells in peritoneal cavity from Mb1creHif1af/f (n = 8), Mb1creHif2af/f (n = 8), and WT mice (n = 9). Data are shown as mean ± s.e.m. Pictures are representative of three independent experiments. NS not significant; *P < 0.05 and **P < 0.01 (two-tailed unpaired Student’s t-test) (see also Supplementary Figure 2).
Fig. 3 HIF-1α deficiency impairs CD1d<sup>hi</sup>CD5<sup>+</sup> B cells IL-10 production and expansion. a Representative plots and quantification of IL-10-producing B cells in bone marrow (BM), spleen (SP), inguinal lymph nodes (ILN), and peritoneal cavity (PerC) from Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup>, Mb<sup>f<sub>t<sub>Hif2a</sub></sup>/f<sup>f<sub>c</sub></sup>, and control mice (n = 3 per group). b Representative plots and absolute numbers of CD19<sup>+</sup>B220<sup>+</sup>CD1d<sup>hi</sup>CD5<sup>+</sup> B cells in spleen from Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> (n = 6) and WT mice (n = 6). c Percentage of BrdU<sup>+</sup> cells in CD1d<sup>hi</sup>CD5<sup>+</sup>, CD1d<sup>lo</sup>CD5<sup>+</sup>, or total splenic B cells isolated from WT (n = 6) and Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> mice (n = 6) 7 days after BrdU treatment. d Representative plots and quantification of IL-10<sup>+</sup>CD1d<sup>hi</sup>CD5<sup>+</sup> B cells in spleen from Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> (n = 7) and WT mice (n = 7). e IL-10 production by sorted CD1d<sup>hi</sup>CD5<sup>+</sup> B cells from Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> (n = 5, 6) and WT mice (n = 5, 6) after stimulation with LPS (left) or anti-IgM (right) for 48 h. Data are shown as mean ± s.e.m. Pictures are representative of three independent experiments. NS not significant; *P < 0.05, **P < 0.01, and ***P < 0.001 (two-tailed unpaired Student’s t-test) (see also Supplementary Figure 3).

HIF-1α deficiency in B cells exacerbates autoimmune diseases. IL-10 production by B cells was previously shown to influence the course of inflammatory autoimmune diseases. Therefore, we hypothesized that HIF-1α in B cells represented a critical node for the modulation of autoimmune diseases. To test this hypothesis, Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> mice were subjected to CIA, a standard murine model of arthritis resembling human rheumatoid arthritis. As shown in Fig. 6a, Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> mice show a significantly increased incidence of arthritis after immunization with collagen II (CII) compared to littermate controls (*P < 0.05, by Kaplan–Meier analysis with log–rank test). The induction of arthritis is dependent on CII immunization, since no clinical symptom is observed in Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> mice without immunization (Fig. 6a, b). Moreover, Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> mice exhibit an earlier disease onset and develop higher clinical arthritis scores than WT mice after CIA immunization (Fig. 6b). Accordingly, Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> mice have an increased paw thickness, synovial inflammation, bone erosions, and number of osteoclasts, confirming an exacerbation of arthritis symptoms in mutant mice (Fig. 6c, d). Similar to TD or TI antibody responses (Supplementary Fig. 2), levels of IgG<sub>1</sub>, IgG<sub>2a</sub>, and IgG<sub>2b</sub> are not changed in Mb<sup>f<sub>t<sub>Hif1a</sub></sup>/f<sup>f<sub>c</sub></sup> and WT arthritic mice (Supplementary Fig. 6a). Next, cytokines mRNA expression pattern was analyzed in synovial tissues. As
expected, an increased level of pro-inflammatory cytokines such as Tnf, Ifng, Il17, Il1b mRNA and a reduced level of Il10 mRNA are detected in synovial tissue of Mb1creHif1af/fo mice compared to WT mice after CIA induction (Fig. 6e, f). Furthermore, the levels of pro-inflammatory cytokines like IL-17 and IFN-γ are higher in Mb1creHif1af/fo mice than WT mice (Fig. 6f, g). Whereas no change in TGF-β levels is detected, the level of IL-10 is reduced in CIA-stimulated splenocytes and splenic B cells from Mb1creHif1af/fo mice (Fig. 6f, g).

To determine whether immune cell populations were altered, Th1 (IFN-γ+CD4+) cells, Th17 (IL-17+CD4+), IL-23R+IL-17+, or GM-CSF+IL-17+ cells, Treg (CD25+Foxp3+CD4+) cells, type 1 regulatory T cells (Tr1) (IL-10+CD4+) cells as well as IL-10+ B cells, ICAM+ B cells, CD73+ B cells, GITRL+ B cells, FasL+ B cells, and PD-L1+ B cells were quantified in the spleen and draining lymph nodes (dLNs) of Mb1creHif1af/fo and WT mice after CIA immunization. Regarding the B cell subsets analyses, there is no difference in ICAM+, CD73+, GITRL+, FasL+, or PD-L1+ B cells in spleen and dLNs (Supplementary Fig. 6b, c), only IL-10+ B cells are detected for the CD25+Foxp3+ Treg population (Fig. 6h, i). In accordance, the suppressive function of Treg cells from Mb1creHif1af/fo or WT mice is similar when co-cultured with
glycoprotein peptide (MOG35-55) induced EAE was applied to mediated by IL-10 production, the myelin oligodendrocyte splenic B cells stimulated with anti-IgM for 8 h. The whole cell lysates were immunoprecipitated with either anti-HIF-1α or anti-pSTAT3 (Ser727) antibodies. ChIP on ChIP assays in enriched splenic B cells pulling down sequentially HIF-1α or anti-pSTAT3 antibodies. HIF-1α and pSTAT3 binding on the HRE regions of Il10 promoter under normoxic or hypoxic conditions for 24 h (n = 3 per group). Data are shown as mean ± s.e.m. Pictures are representative of four (a) or three (c-h) independent experiments. **P < 0.01 and ***P < 0.001 (two-tailed unpaired Student’s t-test) (see also Supplementary Figure 5).

Fig. 5 HIF-1α and STAT3 cooperatively regulate Il10 transcription. a Quantitative RT-PCR analysis of Il10 mRNA expression in WT splenic B cells cultured under normoxia or hypoxia for indicated time (n = 5 per group). b Scheme of Il10 promoter indicating the predicted HRE regions (I, II, III, IV, and V). Enrichment of HIF-1α (c) and H3K4me3 (d) in HRE regions of Il10 promoter in B cells under normoxia or hypoxia for 24 h (n = 3 per group). e Luciferase activity in 293T cells transfected with pGL3 empty vector (EV), HRE constructs (I, II, III, IV, and V) under normoxic or hypoxic conditions for 24 h (n = 3 per group). The Luc/β-gal ratio was normalized to EV at 20% O2. f ChIP assays in enriched splenic B cells showing the recruitment of the endogenous HIF-1α on the HRE regions of Il10 promoter after anti-IgM stimulation for 8 h (n = 3 per group). g Co-immunoprecipitation of HIF-1α and pSTAT3 under normoxic and hypoxic conditions for 24 h (n = 3 per group). h ChIP on ChIP assays in enriched splenic B cells pulling down sequentially HIF-1α and pSTAT3 showing the HIF-1α-pSTAT3 binding on the HRE regions of Il10 promoter after stimulation with anti-IgM for 8 h (n = 3 per group). Data are shown as mean ± s.e.m. Pictures are representative of four (a) or three (c-h) independent experiments. **P < 0.01 and ***P < 0.001 (two-tailed unpaired Student’s t-test) (see also Supplementary Figure 5).

To further confirm the physiological role of HIF-1α in B cells mediated by IL-10 production, the myelin oligodendrocyte glycoprotein peptide (MOG35-55) induced EAE was applied to MbtcreHif1a+/− mice and WT littermates. After immunization with MOG35-55, MbtcreHif1a+/− mice develop higher clinical scores than WT mice (Fig. 7a). Histopathological analyses reveal an increased number of inflammatory foci and demyelination areas in the spinal cords of MbtcreHif1a+/− mice (Fig. 7b). In addition, increased numbers of infiltrated CD4+ T cells, and F4/80+ macrophages are observed in the central nervous system (CNS) of MbtcreHif1a+/− mice compared to WT littermates (Fig. 7c). Like for the CIA model, EAE pathogenesis is associated to Th17, Th1 cells, and production of pro-inflammatory cytokines like IL-17 and IFN-γ. At the peak of EAE disease, increased levels of IL-17 and IFN-γ and a reduced level of IL-10 are found in serum from MbtcreHif1a+/− mice compared to WT littermates (Supplementary Fig. 7a). Furthermore, after in vitro re-stimulation with MOG35-55 for 48 h, splenocytes and splenic B cells from MbtcreHif1a+/− mice produce a reduced level of IL-10 when compared to WT cells (Fig. 7d, e). However, there is no difference in TGF-β and IL-35 production by splenic B cells from MbtcreHif1a+/− mice after re-stimulated with MOG35-55 (Fig. 7e). Next, Th1 (IFN-γ+CD4+) cells, Th17 (IL-17+CD4+, IL-23R+IL-17+), or GM-CSF+IL-17+ cells, Treg (CD25+Foxp3+CD4+) cells, type 1 regulatory T cells (Tr1) (IL-10+CD4+) cells, IL-10+Foxp3+ T cells as well as IL-10+ B cells, ICAM1+ B cells, CD73+ B cells, GITRL+ B cells, FasL+ B cells, and
PD-L1+ B cells were quantified in the spleen, dLNs, and CNS by FACS. No difference is detected in the ICAM+, CD73+, GITRL+, FasL+, and PD-L1+ B cell populations in spleen and dLNs (Supplementary Fig. 7b, c). Th1 and Th17 populations, including pathogenic IL-23R+IL-17+ and GM-CSF+IL-17+ Th17 cells are increased, whereas Tr1, IL-10+Foxp3+ Treg cells, and IL-10-producing B cells are decreased in Mb1creHif1af/f mice compared to WT mice (Fig. 7f–i and Supplementary Fig. 7d), suggesting that HIF-1α expression in B cells limits the progression of EAE by regulating Th1, Th17, and Tr1 cells differentiation. Collectively,

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**CONFLICT OF INTEREST**

The authors declare no competing interests.

**AUTHOR CONTRIBUTIONS**

R.M. designed and performed experiments, analyzed data, and wrote the paper.

J.L. performed experiments and analyzed data.

H.K. and N.H. performed experiments.

K.T. analyzed data.

S.K. performed experiments.

Y.E. and Y.L. generated mouse models.

K.T. and M.N. performed experiments.

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M.N. and M.K. contributed reagents.

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K.T. and T.K. contributed reagents.

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J.K. performed experiments.

K.T. and T.K. contributed reagents.

M.K. performed experiments.

J.K. performed experiments.
our results demonstrate that HIF-1α expression in B cells has a crucial protective function in autoimmune diseases.

**Impaired suppressive function of Hif1a-deficient B cells.** To determine whether the phenotypes of Mb1creHif1af/f mice in autoimmune diseases, including the observed exacerbated pro-inflammatory T cell response, were secondary to a defect in IL-10 production by B cells, naïve CD4 T cells were co-cultured with CD1dhiCD5+ B cells from WT mice in presence or absence of anti-IL-10 antibody. Indeed, CD4 T cells co-cultured with CD1dhiCD5+ B cells in presence of anti-IL-10 antibody shows higher Th1, Th17 and lower Tr1 polarization than the ones cultured in the absence of anti-IL-10 antibody (Fig. 8a). Next, naïve CD4 T cells were co-cultured with CD1dhiCD5+ B cells sorted from Mb1creHif1af/f mice and WT littermates under T cell-polarizing conditions. Naïve CD4 T cells co-cultured with CD1dhiCD5+ B cells from Mb1creHif1af/f mice (CD1dhiCD5+ΔHif1a) show higher polarization into Th1 (IFN-γ+CD4+) and Th17 (IL-17+CD4+) cells than their counterparts co-cultured with sorted CD1dhiCD5+ B cells from WT mice (CD1dhiCD5+WT) (Fig. 8b). These data suggest that the suppressive function of Hif1a-deficient CD1dhiCD5+ B cells on Th1 and Th17 cells differentiation is impaired (Fig. 8b). Interestingly, less Tr1 (IL-10+CD4+) cells are also found when CD4 T cells where co-cultured with Hif1a-deficient CD1dhiCD5+ B cells, whereas no difference is detected in Foxp3+ Treg cells (Fig. 8b, c), suggesting that CD1dhiCD5+ B cells regulate the differentiation of Tr1 cells in a HIF-1α-dependent manner in this culture system, as observed in vivo (Figs. 6 and 7).

This culture system was then used to define whether the impaired regulatory function of Hif1a-deficient CD1dhiCD5+ B cells was due to their defect in IL-10 production. To this end, Hif1a-deficient CD1dhiCD5+ B cells were transduced with an IL-10-overexpression lentivirus (Supplementary Fig. 8a), before co-culturing them with naïve CD4 T cells. Remarkably, the regulatory effects on Th1, Th17, and Tr1 cells differentiation are rescued for IL-10-transduced Hif1a-deficient CD1dhiCD5+ B cells (IL-10-CD1dhiCD5+ΔHif1a) compared to the mock-transduced Hif1a-deficient CD1dhiCD5+ B cells (mock-CD1dhiCD5+ΔHif1a) (Fig. 8b). These data indicate that HIF-1α-dependent IL-10 production is required for the suppressive function of CD1dhiCD5+ B cells on T cells in vitro.

We next used a similar genetic approach to test whether the suppressive defect of Hif1a-deficient CD1dhiCD5+ B cells in autoimmune disease could similarly be rescued by genetically ecotopic-expressing IL-10. Thus, WT and Hif1a-deficient CD1dhiCD5+ B cells were sorted, transduced with mock or IL-10-overexpression lentivirus, and transferred into Mb1creHif1af/f mice that were subsequently immunized with MOG35-55 to induce EAE (Supplementary Fig. 8b). Clinical score and pathological analyses of the spinal cord show that Mb1creHif1af/f mice treated with CD1dhiCD5+(WT) cells have a significantly less severe disease than those treated with CD1dhiCD5+ΔHif1a) cells (**P < 0.05 and ***P < 0.01, by two-way analysis of variance with Bonferroni’s post test and t-test; Fig. 9a, b). Remarkably, Hif1a-deficient CD1dhiCD5+ B cells transduced with the IL-10-overexpression lentivirus (IL-10-CD1dhiCD5+ΔHif1a) ameliorate the disease progression of Mb1creHif1af/f recipient mice as efficiently as CD1dhiCD5+(WT) cells (Fig. 9a, b). Similar results are obtained when analyzing the magnitude of the pro-inflammatory T cell response in these groups of mice. After in vitro re-stimulation with MOG35-55 splenocytes isolated from mice administered with CD1dhiCD5+(WT) or IL-10-CD1dhiCD5+ΔHif1a) cells show a lower level of IL-17, IFN-γ and a higher IL-10 level than the one’s receiving CD1dhiCD5+(ΔHif1a) or mock-CD1dhiCD5+ΔHif1a) cells (Fig. 9c). Accordingly, mice receiving CD1dhiCD5+(WT) or IL-10-CD1dhiCD5+(ΔHif1a) cells have reduced percentage and absolute cell number of Th1 or Th17 populations in dLNs than the mice receiving CD1dhiCD5+(ΔHif1a) or mock-CD1dhiCD5+(ΔHif1a) cells (Fig. 9d). Conversely, increased relative and absolute numbers of Tr1 cells are observed in dLNs from CD1dhiCD5+(WT) or IL-10-CD1dhiCD5+(ΔHif1a) recipient mice compared to CD1dhiCD5+(ΔHif1a) or mock-CD1dhiCD5+(ΔHif1a) recipients (Fig. 9d). Taken together, these findings show that the loss of HIF-1α in B cells causes impaired IL-10 production and aggravating autoimmune diseases (Supplementary Fig. 9).

**Discussion**

Herein, we describe a novel molecular mechanism of immune modulation, which determines the function of IL-10-producing B cells and thereby influences the course of autoimmune disease. We identified HIF-1α as a critical transcriptional factor involved in IL-10 production by B cells, thereby influencing the course of T cell-mediated autoimmune diseases such as EAE and arthritis.

IL-10-producing B cells have been identified as potent players in the inhibition of inflammation in autoimmune disease. Hence, it has been shown that adoptive transfer of B cells taken from DBA/1 mice in the remission phase of arthritis prevents the onset of CIA via the secretion of IL-10. In accordance, transfer of B-cell activating factor of TNF family (BAFF) expanded CD1dhiCD5+ B cells decreased Th17 activation and reduced disease severity of arthritis. In agreement with these data from arthritis models, adoptive transfer of MOG-sensitized CD1dhiCD5+ B cells into WT mice also mitigate the severity of EAE. The immune regulatory function of CD1dhiCD5+ B cells appears to be tightly bound to the production of anti-inflammatory cytokines like IL-10, which limits the immune response to pathogens and thereby prevents damage to the host. Studies have shown that CD1dhiCD5+ B cells have the property to differentiate into plasmablasts after stimulation. Accordingly, we found that CD4fiCD138fi plasmablasts are also reduced in Hif1a-deficient mice.
mice after EAE induction, suggesting that loss of HIF-1α causes impaired CD1d<sup>+</sup>CD5<sup>+</sup> B cells and increases likelihood to develop autoimmune disease. Published studies have shown that calcium sensor stromal interaction molecules (STIM) and IL-21-dependent cognate interactions are required for the function of IL-10-producing B cells<sup>32,33</sup>. However, the molecular regulation of IL-10 production in B cells was incompletely defined to date. Our data now show that HIF-1α is crucial in inducing IL-10 production by B cells. Lack of HIF-1α in B cells causes reduced IL-10 production followed by enhanced Th17 cells. In addition, increased IL-17 and IFN-γ production in Hif1a-deficient mice is associated with a
strong exacerbation of EAE and inflammatory arthritis. HIFs have been previously suggested to influence adaptive and innate immunity. Differential effects of HIF-1α and HIF-2α have previously been shown in immune cells. However, the roles of HIF-1α and HIF-2α in B cells have not been shown. Our data suggest that despite the description of hypoxic niches in the bone marrow and regions within the spleen, HIF-1α and HIF-2α appear non-essential for the development of B cell subsets in the bone marrow as well as spleens and lymph nodes in homeostasis. However, LPS or BCR-mediated activation of B cells causes massive induction of HIF-1α, but not HIF-2α, in an oxygen-independent way. While in macrophages, HIF-1α accumulation requires NF-κB-dependent transcriptional event. HIF-1α in BCR-stimulated B cells is induced via the ERK-STAT3 signaling pathway. After B cell activation, STAT3 is phosphorylated at position Ser727, but not at the Thr705 site. Our data show that phosphorylated-STAT3 is then effectively induced Hif1a gene transcription in activated B cells. Our findings indicate that HIF-1α contributes to CD1d hiCD5+ B cell proliferation and IL-10 production. Emerging studies indicate that metabolism is important for B cell function and proliferation. In accordance with other publications, we found that CD1d hiCD5+ B cells have a higher glycolytic activity compared to CD1d loCD5−B cells, which can control the normal expansion of CD1d hiCD5+ B cells. Our study shows for the first time that this glycolytic metabolism is dependent on HIF-1α expression. In addition, HIF-1α effectively binds to the IL10 promoter at two HRE elements, which also correlates with the actively transcribing regions. Since previous studies have shown that proximal broad H3K4me3 domains are highly dynamic in different cell types and conditions, the high enrichment of H3K4me3 in HRE I or HRE II regions is probably related to the hypoxic condition. We show that HIF-1α transcriptionally enhances IL10 mRNA expression. In line with our results, it has been shown that both IL-21 and IL-27 induce ERK and STAT3 activation and upregulate IL-10 in CD4 T cells. Notably, HIF-1α appears to specifically induce IL-10 in B cells, as other immune regulatory factors expressed in B cells, such as TGF-β and IL-35, are not altered in Hif1a-deficient B cells. In accordance with our findings, the HIF-1α-dependent regulation of IL-10 has been previously reported in macrophages, T cells, and myocytes using different approaches of HIF-1α knockdown. Our data also show that manipulation of HIF-1α expression in B cells influences T cells. T cells have been intimately associated with IL-10-producing B cells as they drive the differentiation of cognate B cells into IL-10-producing B cells, suggesting a mutual regulation between IL-10-producing B cells and T cells. In accordance with that, our data show that impaired IL-10 production by HIF-1α-dependent B cells is associated with decreased T cell levels, indicating a regulatory network between IL-10-producing B cells and T cells.

Therapeutically, fostering of HIF-1α expression may provide a tool to increase IL-10-producing B cells and limit autoimmune diseases such as EAE and arthritis. Inhibitors of prolyl-hydroxylases (PHD), for instance, are agents that can induce HIF-1α. Treatment with PHD inhibitors has been shown to ameliorate endotoxic shock as well as inflammatory bowel disease in mice. In these studies, PHD inhibitors enhanced the numbers of IL-10-producing B cells and reduced expression of inflammatory cytokines. Therefore, activating the HIF-1α axis through pharmacologic agents may indeed provide a tool to augment the immune regulatory potential of IL-10-producing B cells with the potential to prevent and/or treat systemic autoimmune inflammatory diseases.

In summary, we provide a novel molecular mechanism for the regulation of autoimmune disease by CD1d hiCD5+ B cells. By modulating glycolytic metabolism, HIF-1α regulates CD1d hiCD5+ B cell expansion. Moreover, we identified HIF-1α as a critical node involved in IL-10 production by B cells. HIF-1α effectively binds to hypoxia response elements on the IL10 promoter, resulting in expression of IL-10 in B cells. In consequence, HIF-1α expression in B cells regulates autoimmune diseases such as EAE and arthritis.

**Methods**

**Mice.** C57BL/6 WT mice (027) were purchased from Charles River Laboratories (Sulzfeld, Germany). To generate B cell-specific Hif1α or Hif2α-deficient mice, Hif1α flox/flox mice or Hif2α flox/flox mice were crossed with Mb1-cre mice. Hif1α flox/flox mice, Hif2α flox/flox mice, and Mb1-cre mice were previously described.

The mice were bred and maintained on a C57BL/6 background and Hif1α−/− or Hif2α−/− mice were genotyped by PCR. Sex- and age-matched (8–10 weeks) mice were killed using CO2 and terminated via cervical dislocation for in vitro and ex vivo experiments. Animals were kept in a specific pathogen-free facility and animal experiments were approved by local ethics committee of Regierung von Mittelfranken (license AZ: 55.2 2532-2-198 and 55.2 DMS-2532-2-94), Germany.

**Flow cytometry and cell sorting.** Single-cell suspensions were prepared from bone marrow (femurs), spleen, inguinal lymph nodes, peritoneal cavity, and peripheral blood. Dead cells were lysed with ammonium-chloride-potassium (ACK) buffer. Cells were Fc-blocked (CD16/CD32) and stained with antibodies (Supplementary Table 1). Analyses of the expression of cell surface molecules on a single cell level were performed by flow cytometry with Calibur (BD) or Cytoflex (Beckman Coulter) flow cytometer. Dead cells were detected using a LIVE/DEAD Fixable Violet Dead Cell Stain Kit (L34955, Life Technologies) before cell surface staining. For analysis of intracellular IL-10 expression by B cells, LPS, phosphor-12- myristate-13-acetate (PMA), ionomycin, and monensin (IL-PI4) were added to the cultures 5h before fixation and permeabilizing with the Foxp3 Transcription Factor Staining Buffer Stainer Kit (00/5523/00, ebiosciences) according to the manufacturer’s instruction. All flow cytometry experiments were gated on viable, single lymphocytes and data were analyzed with FlowJo software (Treestar).

For cell sorting, CD1d−/−CD5+CD19+ B220+ and CD1d+CD5+CD19+ B220+ B cells were sorted from splenocytes using a MoFlo cell sorter (DAKO instruments). Cell purity of 98–99% was generally achieved.

Gating strategies are presented in Supplementary Fig. 10.

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**Fig. 7** HIF-1α deficient mice show exacerbated experimental autoimmune encephalomyelitis. a Clinical score of Mbp14−/−Hif1fl/fl (n = 10) and WT mice (n = 10) immunized with MOG35-55. b Histopathology sections and quantifications of inflammatory loci (arrows) and demyelinated area (dashed line) in the spinal cord of Mbp14−/−Hif1fl/fl (n = 10) and WT mice (n = 10) showing lymphocyte infiltration (H&E) and demyelination area (LFB). Scale bars, 500 μm. c Representative plots and quantification of CNS-infiltrating cells in Mbp14−/−Hif1fl/fl (n = 7) and WT mice (n = 7) 18 days after inductions of EAE. d IL-17, IFN-γ, IL-10, and TGF-β expression by splenocytes isolated from mice as described in c followed by an in vitro re-stimulation with MOG35-55 (10 μM) for 48 h.

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**Fig. 8** HIF-1α expression in B cells regulates helper T cell polarization in vitro. 

**a** Naïve CD4 T cells isolated from WT mice were co-cultured with CD1d<sup>+</sup>CD5<sup>+</sup> B cells from WT mice with anti-IL-10 antibody or isotype control antibody at a 1:1 T/B ratio in a trans-well system for 3 days under T cell-polarizing conditions. Percentage of IFN-γ<sup>+</sup>CD4<sup>+</sup> (Th1) cells, IL-17<sup>+</sup>CD4<sup>+</sup> (Th17) cells, and IL-10<sup>+</sup>CD4<sup>+</sup> (Tr1) cells were determined (n = 3 per group).

**b** Naïve CD4 T cells isolated from WT mice were co-cultured with CD1d<sup>+</sup>CD5<sup>+</sup> B cells from WT mice (CD1d<sup>+</sup>CD5<sup>+</sup> (WT)), Mb1creHif1af/f mice (CD1d<sup>+</sup>CD5<sup>+</sup> (ΔHif1a)), Mb1creHif1af/f mice after transduction of pDBR lentivirus (mock-CD1d<sup>+</sup>CD5<sup>+</sup> (ΔHif1a)), or Mb1creHif1af/f mice after transduction of pDBR-IL-10 lentivirus (IL-10-CD1d<sup>+</sup>CD5<sup>+</sup> (ΔHif1a)) at a 1:1 T/B ratio in a trans-well system for 3 days under T cell-polarizing conditions. Percentage of IFN-γ<sup>+</sup>CD4<sup>+</sup> (Th1) cells, IL-17<sup>+</sup>CD4<sup>+</sup> (Th17) cells, and IL-10<sup>+</sup>CD4<sup>+</sup> (Tr1) cells were determined (n = 3 per group).

**c** Naïve CD4 T cells isolated from WT mice were co-cultured with CD1d<sup>+</sup>CD5<sup>+</sup> B cells from WT mice (CD1d<sup>+</sup>CD5<sup>+</sup> (WT)) or Mb1creHif1af/f mice (CD1d<sup>+</sup>CD5<sup>+</sup> (ΔHif1a)) at a 1:1 T/B ratio in a trans-well system for 3 days. Percentage of IL-10<sup>+</sup>CD4<sup>+</sup> T cells and Foxp3<sup>+</sup>CD4<sup>+</sup> T cells were determined (n = 6 per group). Data are shown as mean ± s.e.m. Pictures are representative of three independent experiments. NS not significant; *P < 0.05, **P < 0.01, and ***P < 0.001 (unpaired, two-tailed Student’s t-test)
Representative of three independent experiments. NS not significant.

Bonferroni

CD1dhiCD5+(ΔHif1a)

Fig. 9 Aggravating EAE in Hif1a-deficient mice is rescued by ectopic expression of IL-10 in Hif1a-deficient CD1dhiCD5+(WT) cells. a Clinical score of MbFrt/hif1a/Δ mice immunized with MOG35-55 after adoptive transfer of CD1dhiCD5+(WT) cells from WT mice (CD1dhiCD5+(WT)) (n = 5), MbFrt/hif1a/Δ mice after transduction of pDBR lentivirus (Mock-CD1dhiCD5+(WT)) (n = 5), or MbFrt/hif1a/Δ mice after transduction of pDBR-IL-10 lentivirus (IL-10-CD1dhiCD5+(WT)) (n = 5). b Pathophysiological pictures and quantifications of inflammatory loci (arrows) and demyelinated area (dashed line) in the spinal cord sections from MbFrt/hif1a/Δ mice immunized with MOG35-55 after adoptive transfer of CD1dhiCD5+(WT) (n = 5), CD1dhiCD5+(ΔHif1a) (n = 6), mock-CD1dhiCD5+(ΔHif1a) (n = 5), or IL-10-CD1dhiCD5+(ΔHif1a) cells (n = 5). Scale bars, 500 μm. c Representative plots and quantification of IL-17+CD4+(Th17), IFN-γ+CD4+(Th1), IL-10+CD4+(Th17), IL-22+Foxp3+CD4+(Treg) cells in draining lymph nodes from MbFrt/hif1a/Δ mice immunized with MOG35-55 after adoptive transfer of CD1dhiCD5+(WT) (n = 5), CD1dhiCD5+(ΔHif1a) (n = 6), mock-CD1dhiCD5+(ΔHif1a) (n = 5), or IL-10-CD1dhiCD5+(ΔHif1a) cells (n = 5). Data are shown as mean ± s.e.m. Pictures are representative of three independent experiments. NS not significant; *P < 0.05, **P < 0.01, and ***P < 0.001 (two-way analysis of variance with Bonferroni’s post-test (a) or two-tailed unpaired Student’s t-test (b–d)) (see also Supplementary Figure 8)
B cells isolation and stimulation. For B cells isolation, rest B cells were purified from spleen by negative selection with anti-CD43 magnetic beads or positive selection with anti-CD43 magnetic beads (Miltenyi Biotec) following the manufacturer’s instructions. The purified B cells population was >95% B220 positive cells. B cells were cultured in RPMI supplemented with 10% FCS, 2-ME, penicillin (100 U/mL), and streptomycin (100 μg/mL). Purified B cells (2×10^6/mL) were cultured with LPS (10 μg/mL) or IgM-specific goat F(ab')2 antibody (10 μg/mL) in a 48-well flat-bottom plate.

Quantitative PCR analysis. Total cell or tissue RNA was extracted using Trizol reagent (Invitrogen) and complementary DNA was synthesized by using High-Capacity CDNA Reverse Transcription Kit (4368814, Thermo Scientific) according to the manufacturer’s instructions. Quantitative PCRs (qPCRs) were performed using SYBR Green I-dTTP (Eurogentec). Specific primers used for qPCR are listed in Supplementary Table 2. The levels of Hif1α, Hif2α, Tgfβ, Il1β, P35, Ebf3, Hif1α, Hif2α, Il17, Igf1, Il1b, Glut, Pkm2, H2k, Ldha, Pdk1, and Gpi1 were determined by evaluating the threshold cycle (Ct) of target gene after normalization against the Ct value of Hprt and calculated using the formula 2^(-ΔΔCt) (of target gene-Ct of Hprt).

Western blot and co-immunoprecipitation analysis. Cultured B cells were washed twice with PBS and homogenized into interaction buffer (8 μM urea, 10% glycerol, 1% SDS, 10 mM Tris-HCl pH 6.8, protease inhibitor complete (Roche), 1 mM Sodium-Vanadate). Total cell lysates were resolved on 10% SDS-PAGE and were transferred to nitrocellulose membrane (Bio-Rad). The following primary antibodies were used (Supplementary Table 1): HIF-1α antibody, HIF-2α antibody (Novus), STAT3 antibody, phospho-ERK antibody, total ERK antibody, phosphorylated ERK antibody, total ERK antibody (Cell Signaling), and β-actin antibody (Sigma). The western blot bands were quantified using ImageJ Software.

The Dynabeads-immunoprecipitation kit (14321D, Invitrogen) was used for the endogenous co-immunoprecipitation assay and nuclear extracts preparation as described previously5. Briefly, splenic B cells were enriched from WT mice and stimulated with anti-IgM (10 μg/ml) for 8 h. Five milligrams of nuclear extracts were incubated with 5 μg of anti-HIF-1α antibody (H1667), anti-pSTAT3(727) antibody, or control IgG with Dynabeads protein G according to the manufacturer’s instruction. Immunoblots were then performed using HIF-1α or anti-STAT3 phosphorylated at Ser727 as described above. Full size images are presented in Supplementary Fig. 11.

Luciferase reporter assay. HRE regions (1–V) of Il10 promoter (Supplementary table 2) were amplified by PCR from genomic DNA extracted from splenocytes in C57BL/6 WT mice and cloned into the pGL3 firefly reporter vector (Promega). 293T cells were co-transfected with luciferase reporter construct and β-gal plasmid using Lipofectamine 2000 (invitrogen). Transfected cells were cultured under normoxic (21% O2) or hypoxic (1% O2) conditions for 24 h. Cells were then lysed and luciferase activity was quantified and normalized to the activity of the co-transfected β-gal reporter gene.

RNA interference. STAT3, ERK, and RdA, or scrambled siRNA lentivirus constructs were obtained from Applied Biological Materials (ABM, Richmond, BC, Canada). Transduced splenic B cells were incubated with 10 μg/ml anti-IgM or LPS for 4 h and then cell lysates was used for western blot.

Chromatin immunoprecipitation. Splenic B cells were enriched from C57BL/6 WT mice and cultured in medium alone or anti-IgM (10 μg/ml) for 8 h. Next, ChIP experiments were performed with ChIP-IT Express kit (35018, Active Motif) according to the manufacturer’s protocol. Ten micrograms of anti-pSTAT3(727) antibody, or anti-HIF-1α, or anti-HIF-2α antibodies, as well as control IgG antibody, were used for the immunoprecipitation. Primer sequence for STAT3 binding site was described previously (Supplementary Table 2). HIF-1α binding sites were predicted by JASPAR with the consensus core (A/GCGTG) and primers were designed by Primer-BLAST (Supplementary Table 2).

Glucose uptake and lactate production assays. Glucose uptake was determined using Glucose uptake cell-based assay kit (600470, Cayman) according to the manufacturer’s protocol.

Histology. On day 45 of CIA model, whole paw joints were fixed in 4% paraformaldehyde, decalified in EDTA, and then embedded in paraffin. Specimens were longitudinally cut into 4 μm sections, then hematoxylin and eosin (H&E) and tartrate-resistant acid phosphatase (TRAP) stainings were performed. Analysis of epitrochanteral and femoral head necrosis was performed using unbiased cell counting method. All images were taken using a microscope (Olympus) and processed using ImageJ software. The number and size of osteoclasts were counted using the ImageJ software. For necrosis scoring, sections were classified as 0, no necrosis; 1, minimal; 2, mild; 3, moderate; 4, severe; 5, extensive.

Isolation of CNS-infiltrated lymphocytes. After cardiac perfusion with PBS, CNS tissues were digested with 2.5 mg/ml collagenase D (Roche) and 1 mg/ml DNase (Roche) for 2 h at 37 °C. Isolated lymphocytes were resuspended in the tissue through 70 μm cell strainers, followed by Percoll (Millipore) gradient (70%/37%) centrifugation. Lymphocytes were collected from the interface and washed in PBS.
Lentivirus transfection and adoptive transfer. Production of viral supernatants and B cell transduction were described previously. Briefly, lentiviral particles were produced in 293T cells by co-transfection of psPax2 packaging vector (Addgene), VSVG envelope plasmid (Addgene), pMDR (mock), or pMDR-IL-10 plasmid using Lipofectamine 2000 (Invitrogen). After 48 h, supernatants were collected, filtered (0.45 μm), and supplemented with 10 nM HEPS (Invitrogen) and 10 μg/ml polybrene (Millipore). Sorted CD1dhiCD5+ B cells were centrifuged at 3.5 × 10^7/well in six-well plates at 4 ml of viral supernatants in a total volume of 4 ml at 1800 rpm during 75 min at room temperature, and then washed in PBS. A total of 1.5 × 10^6 transduced CD1dhiCD5+ B cells were transferred intravenously into recipient mice 24 h before EAE induction.

Statistical analysis. For comparison of the two groups, linear regression with a 95% confidence interval, and two-tailed Student’s t-test were used. Kaplan–Meier analysis with log-rank test was used to determine the significance of CIA incidence. Two-way ANOVA of variance with Bonferroni’s post test for paired data was used to determine the significance of EAE clinical scores in adoptive transfer experiment. GraphPad Prism software 6.0 was used for statistical analysis. P-value of less than 0.05 was considered statistically significant.

Data availability. The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the authors on request.

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Author contributions

X.M. and A.B. designed the study and wrote the manuscript; X.M., B.G., and K.X.K. carried out the in vitro experiments; X.M. and Y.L. carried out the in vivo experiments; M.S.W., X.-X.C., J.J., and S.F. contributed to discussion and manuscript preparation; G.S. and A.B. supervised the study and edited the manuscript.