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ORIGINAL ARTICLE

Hypoxia-inducible factor-1α regulates the interleukin-6 production by B cells in rheumatoid arthritis

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Abstract

Objectives. Rheumatoid arthritis (RA) is a disease characterised by bone destruction and systemic inflammation, and interleukin-6 (IL-6) is a therapeutic target for treating it. The study aimed at investigating the sources of IL-6 and the influence of hypoxia-inducible factor-1α (HIF-1α) on IL-6 production by B cells in RA patients. **Methods.** The phenotype of IL-6-producing cells in the peripheral blood of RA patients was analysed using flow cytometry. Bioinformatics, real-time polymerase chain reaction, Western blot and immunofluorescence staining were used to determine the IL-6 production and HIF-1α levels in B cells. A dual-luciferase reporter assay and chromatin immunoprecipitation were used to investigate the regulatory role of HIF-1α on IL-6 production in human and mouse B cells. **Results.** Our findings revealed that B cells are major sources of IL-6 in the peripheral blood of RA patients, with the proportion of IL-6-producing B cells significantly correlated with RA disease activity. The $CD27^{-}IgD^{+}$ naïve B cell subset was identified as the typical IL-6-producing subset in RA patients. Both HIF-1α and IL-6 were co-expressed by B cells in the peripheral blood and synovium of RA patients, and HIF-1α was found to directly bind to the *IL6* promoter and enhance its transcription. **Conclusion.** This study highlights the role of B cells in producing IL-6 and the regulation of this production by HIF-1α in patients with RA. Targeting HIF-1α might provide a new therapeutic strategy for treating RA.

Keywords: B cells, HIF-1α, IL-6, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, resulting in cartilage and bone damage, and even disability.[1] The activation of the immune and nonimmune systems is indispensable for the initiation, progression and relapse of this debilitating disease.[2–4] Effector $CD4^+$ T cells, such as Th17 cells, T follicular helper cells (Tfh) and regulatory T cells (Treg), have been extensively investigated in RA pathogenesis.[5–8] However, the roles of B cells are not fully understood and are sometimes controversial. Plasma cell-secreted auto-antibodies, such as rheumatoid factors (RFs) and anticyclic citrullinated peptide antibodies (ACPAs), correlate with disease activity.[1,4] B cells that produce the receptor activator of nuclear factor kappa-B ligand (RANKL) have a pro-inflammatory role in RA synovium.[9] Nevertheless, B cells also produce IL-8 and IL-10 that alleviate arthritis.[10–12] These facts imply the complicated roles of B cells in RA, which need to be elucidated.

An inflamed synovial joint is considered a micro-environment with profound hypoxia,[13] associated with increased activity of signalling pathways including hypoxia-inducible factor 1α (HIF-1α), NF-κB and STAT3.[14] In response to the low O_2 environment, HIF-1α plays a crucial role in regulating cell function, metabolism and vascular adaptation.[15] For instance, HIF-1α-dependent glycolysis is essential during the differentiation of pro-inflammatory macrophages (M1 subtype).[16] HIF-1α also promotes the expansion of Th17 and Tfh cells, while inhibiting Treg differentiation, indicating its selective modulation of $CD4^+$ T cell subset homeostasis.[17,18] Furthermore, *Hif1a*-deficient mice exhibit a remarkably elevated level of serum rheumatoid factor (RFs) and abnormal B cell development.[19] B cell-specific deletion of HIF-1α leads to impaired IL-10 production and exacerbated arthritis development in mice, pointing to a protective role of B cell-derived HIF-1α in arthritis.[11] However, the conflicting results prompt further exploration of HIF-1α's selective regulation of B cell (effector and regulatory) subset responses.

HIF-1α is not only critical for IL-10 expression but also plays a role in the production of pro-inflammatory cytokines, such as IL-6,[14] which is a pleiotropic cytokine that regulates both immune and nonimmune cells in RA patients.[20] The correlation between upregulated serum IL-6 level and the phosphorylation of STAT3 in $CD4^+$ T cells as well as the disease activity (DAS28) of RA has been noted.[6] The enhancement of the IL-6-pSTAT3 signalling pathway alters the balance between Tfh cells and T follicular regulatory T cells (Tfr)[21] and is linked to the activation of B cells in RA patients.[22] In collagen-induced arthritis (CIA) mouse model, synovial fibroblast-derived IL-6 converts $Foxp^{3+}$ Treg cells into IL-17- and RANKL-producing Th17 cells.[23] These findings highlight the pathogenic role of IL-6 in RA. However, the source of IL-6-producing cells and the regulatory role of HIF-1α in IL-6 production in RA patients remain elusive.

Previous research has shown that B-cell-derived IL-6 is critical for systemic inflammation in mouse lupus.[24] In this study, we have demonstrated that B cells are a major source of IL-6 in the peripheral blood of RA patients and that IL-6 production by B cells is regulated by HIF-1α at the transcriptional level. Given these findings, targeting HIF-1α may serve as a novel therapeutic target in RA.

RESULTS

B cells are one of the major sources of IL-6 in the peripheral blood of RA patients and are correlated with RA disease activity

Serum IL-6 levels are increased and correlated with DAS28 in patients with RA.[22] However, the source of IL-6 in the peripheral blood of RA patients remains undefined. To this end, flow cytometry was applied to identify IL-6-producing cell subsets in the peripheral blood of RA patients (Supplementary figure 1a). Intriguingly, $CD19^+$ B cells had the highest ratio (median = 10.5%) of IL-6-producing cells when compared to $CD14^+$ monocytes (median = 4.4%), $CD4^+$ T cells (median = 2.8%), $CD56^+$ NK cells (median = 1.8%) and $CD8^+$ T cells (median = 0.8%; Figure 1a). Although $CD14^+$ monocytes have the highest ratio (median = 12.5%) of $IL\text{-}6^+$ cells in healthy donors, the proportion of IL-6-producing monocytes in the peripheral blood of RA patients was significantly decreased (0.32-fold, $P = 0.0007$). In addition, IL-6-producing cell ratios were significantly increased in $CD19^+$ B cells (1.61-fold, $P = 0.0015$), $CD4^+$ cells (1.96-fold, $P = 0.0008$) and $CD56^+$ NK cells (2.48-fold, $P < 0.0001$) of RA patients compared with healthy controls (HCs; Figure 1b). When comparing

the number of IL-6-producing cell types, $CD4^+$ cells and $CD19^+$ B cells were the most abundant ones in RA (Figure 1c). We then analysed the relationship between the IL-6-producing cell ratio and RA-associated clinical data. Strikingly, only the ratio of $CD19^+IL\text{-}6^+$ cells positively correlated with C-reactive protein (CRP) levels ($P = 0.03$), RF (IgM) levels ($P = 0.02$) and RF (IgG) levels ($P = 0.04$; Figure 1d and Supplementary figure 1d–f). Moreover, we separated the RA patients into two groups: nontreatment and antirheumatic treatment groups. We found no difference in the percentages of IL-6-producing B cells ($P = 0.73$). B cells are the primary producers of IL-6 in both the nontreatment group and the antirheumatic treatment group. Collectively, these data suggest that B cells are one of the major sources of IL-6 in the peripheral blood of RA, and the IL-6-producing B cells correlate with RA disease activity.

Next, we sought to elucidate the immune phenotype of the IL-6-producing B cells. First, we analysed the expression levels of B cell-specific markers, such as IgD, CD27 and CD38, in IL-6-producing B cells of five drug-free RA patients (Supplementary figure 1b). The heatmap of multiple parameter analysis showed that $CD19^+IL\text{-}6^+$ cells had high levels of IgD and CD20, median levels of CD24 and CD38, and low levels of CD27 and CD11c (Supplementary figure 1c). We then checked the IL-6 production in classically defined B cell subsets: $CD20^-CD38^+$ plasmablasts (PB), $CD20^+CD24^+CD38^+$ transitional B cells (tB), $CD20^+CD27^-IgD^+$ naïve B cells (Bn), $CD20^+CD27^+IgD^+$ nonclass switch memory B cells (ncsBm), $CD20^+CD27^+IgD^-$ class switch memory B cells (csBm) and $CD20^+CD11C^+CD27^-$ atypical memory B cells (aBm; Supplementary figure 1g).[25] The percentages of $IL\text{-}6^+$ cells in ncsBm (median = 24.5%) or Bn (median = 19.4%) were much higher than those of other subclusters, but Bns had the highest number of $IL\text{-}6^+$ cells (median = 5.2×10^3 cells/million; Figure 1e). These data demonstrated that the $CD20^+CD27^-IgD^+$ naïve B cell is the primary source of IL-6 production among B cells in the peripheral blood of patients with RA.

HIF-1α in B cells correlates with the IL-6 production and DAS28 in RA patients

We investigated the expression of IL-6 in naïve B cells and plasmablasts in healthy individuals using GEO microarray data (GSE42724).[26] We found that *IL6* mRNA was significantly higher in naïve $CD20^+CD27^-IgD^+$ B cells than in plasmablasts, which is consistent with our finding on the protein level (Supplementary figure 2a).

To further understand the regulation of IL-6 production in naïve B cells, we performed KEGG enrichment analysis based on the significantly upregulated genes in naïve B cells. We found that NF-κB, HIF-1α and JAK–STAT signalling were significantly enriched in naïve B cells (Supplementary figure 2b), which is consistent with the activation of HIF-1α, NF-κB and STAT3 in patients with RA.[13,14,27] We also built a protein–protein interaction network using the *String* tool (https://string-db.org) based on the upregulated genes of naïve B cells (Supplementary figure 2c), which revealed IL6 as a central node. We further used the *EcCentricity* algorithm to identify the top 10 hub genes, including *IL6*, *IL4R*, *STAT6* and *HIF1A*, in this interaction network (Supplementary figure 2d). Comparing the expression of transcription factors in the two B cell subsets revealed that *HIF1A*, *EBF1*, *STAT6* and *PAX5* were much higher in $CD27^-IgD^+$ Bn cells, while *XDP1* and *PRDM1* were mainly expressed in plasmablasts (Figure 2a). These results suggest that HIF-1α may be critical to naïve B cells' function, such as IL-6 expression.

In order to investigate the role of HIF-1α in RA patients, we analysed the expression of HIF-1α in B cells of RA patients by flow cytometry. Our results revealed that the level of HIF-1α in B cells exhibited a positive correlation with DAS28, which is the disease activity score of RA ($P = 0.0479$; Figure 2b). The co-staining analysis further confirmed the existence of $IL\text{-}6^+$ HIF-$1\alpha^+$ B cells in the peripheral blood of RA patients (Figure 2c). Moreover, we observed that the ratio of $IL\text{-}6^+$ B cells was not only positively correlated with the ratio of HIF-$1\alpha^+$ B cells ($P = 0.0095$) but also with HIF-1α levels (GMFI) in B cells ($P = 0.025$; Figure 2d, e). Finally, we detected $CD19^+IL\text{-}6^+HIF\text{-}1\alpha^+$ triple-positive cells in the synovium of patients with RA (Figure 2f, Supplementary figure 2e). Taken together, we conclude that HIF-1α in B cells correlates with the disease activity of RA patients and IL-6 expression.

HIF-1α regulates IL-6 expression in human B cells

As HIF-1α plays a crucial role as a transcriptional factor in regulating inflammation and immunity,[28] we hypothesised that it might also regulate IL-6 production in B cells. To test this hypothesis, we

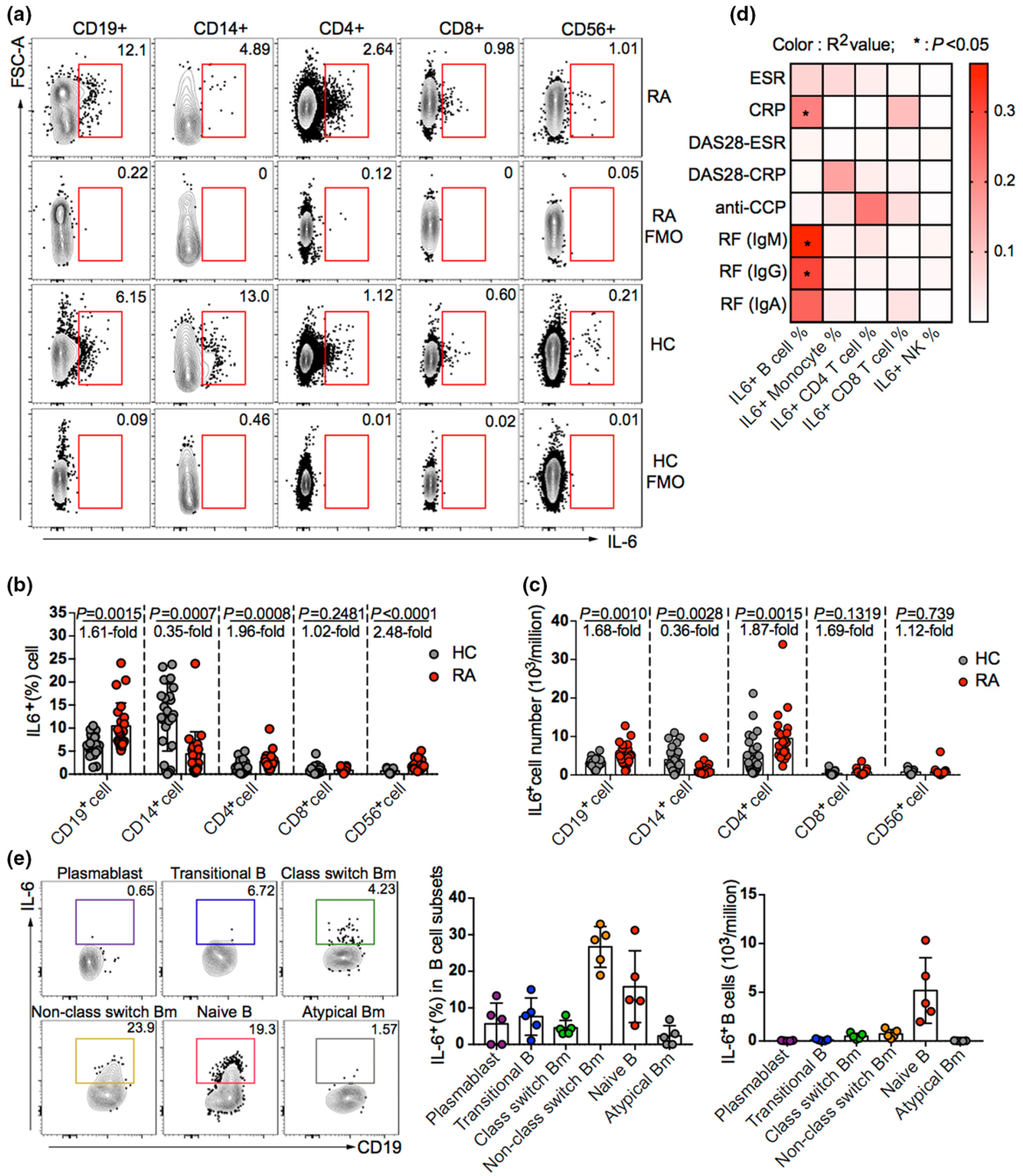

Figure 1. B cell is the major source of IL-6 and correlates with disease activity in patients with rheumatoid arthritis (RA). **(a)** Representative plots of flow cytometric analysis for the IL-6-producing cell subsets ($CD19^+$ B cells, $CD14^+$ monocytes, $CD4^+$ T cells, $CD8^+$ T cells, and $CD56^+$ NK cells) in the peripheral blood of RA patients and healthy individuals. PBMCs were stimulated by PMA (25 ng mL^{-1}) and ionomycin (1 μg mL^{-1}) for 6 h and brefeldin A (10 μg μg mL^{-1}) was added at the last 4 h. IL-6-APC FMO control was used to gate out the IL-6-positive cells. **(b)** Quantification and comparison of IL-6-producing cell types (ratios) in the peripheral blood of RA patients (n = 25) and healthy individuals (n = 23). **(c)** Quantification and comparison of IL-6-producing cell types (number) in the peripheral blood of RA patients (n = 25) and healthy individuals (n = 23). **(d)** Heat map of correlation analysis between IL-6-producing cell types and RA-related clinical factors. Spearman's correlation analysis was used. $P < 0.05$ was considered significant. Colour represents the square value of the relevant score (R^2). *$P < 0.05$. **(e)** Representative plots and quantification of flow cytometric analysis comparing the IL-6-producing B cell subsets ($CD19^+CD20^-CD38^+$ Plasmablasts, $CD19^+CD20^+CD38^+$ $CD24^+$ transitional B cells, $CD19^+CD20^+IgD^-CD27^+$ class switch memory B cells, $CD19^+CD20^+IgD^+CD27^+$ non-class switch memory B cells, $CD19^+CD20^+IgD^+CD27^-$ naïve B cells, $CD19^+CD20^+CD11c^+CD27^-$ atypical memory B cells) in the peripheral blood of drug-free RA patients (n = 5).

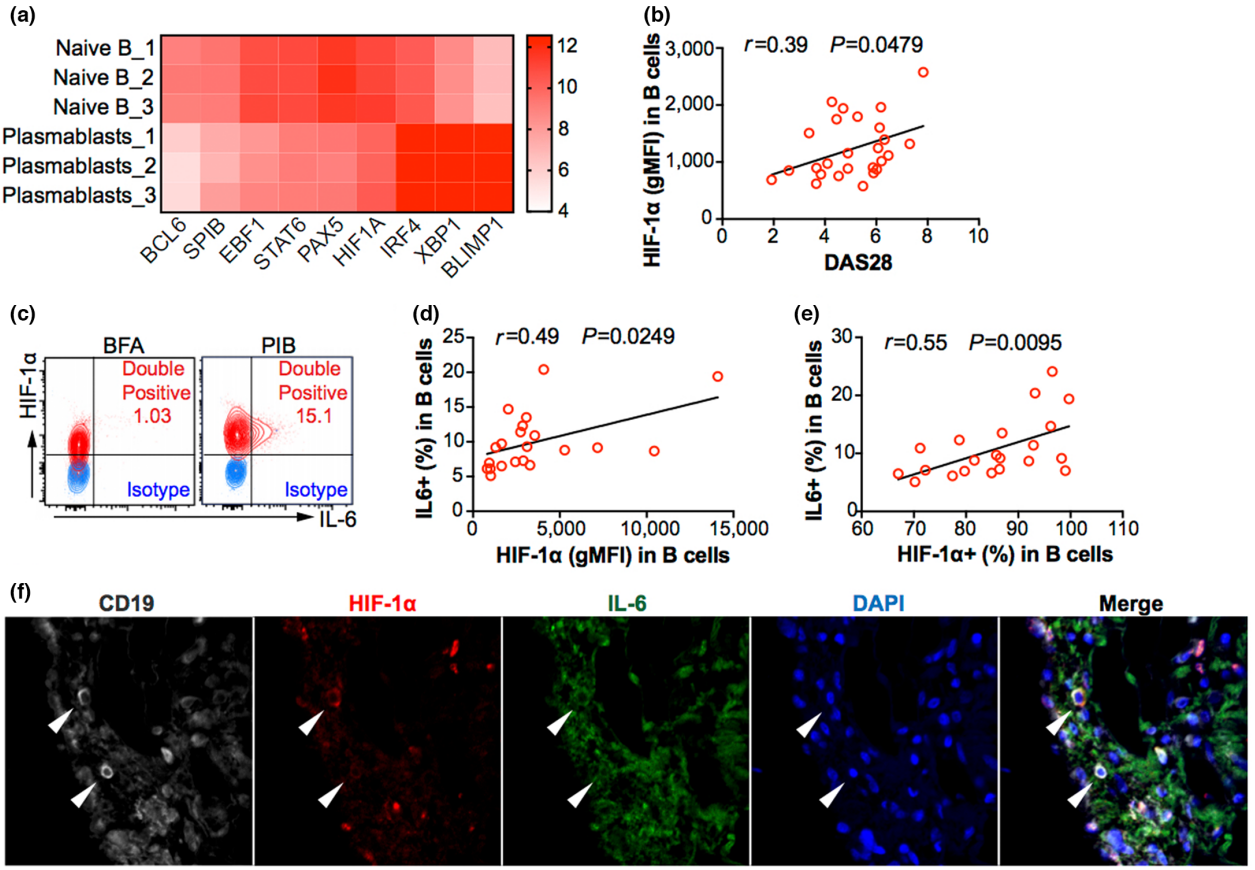

Figure 2. HIF-1α in B cells correlates with DAS28 and IL-6 production. **(a)** Heat map of selected differentially expressed transcription factors between naïve B cells and plasmablasts of healthy individuals (n = 3). The microarray data were downloaded from the GEO dataset (GSE42724) and re-analysed by *R*. **(b)** Correlation curve of the HIF-1α level in B cells (gMFI) and the disease activity score (DAS28) of RA patients (n = 26). Spearman's correlation analysis was used. $P < 0.05$ was considered significant. **(c)** Representative plots of flow cytometric analysis for IL-6 and HIF-1α expression in B cells from the peripheral blood of RA patients (Blue plots represent the control stained with isotype Ab of anti-HIF-1α, and without anti-IL-6; Red plots represent the test sample stained with anti-HIF-1α and anti-IL-6 florescence Abs). Left: only add brefeldin-A for 4 h as control; Right: PMA and Ionomycin stimulation for 6 h and add brefeldin-A for the last 4 h. **(d, e)** Correlation curve of $CD19^+IL\text{-}6^+$ ratio and $CD19^+$ HIF-$1\alpha^+$ ratio (D), $CD19^+IL\text{-}6^+$ ratio and $CD19^+$ HIF-$1\alpha^+$ GMFI (E) in RA patients. Spearman's correlation analysis was used. $P < 0.05$ was considered significant. **(f)** Immunofluorescence staining for CD19 (grey), HIF-1α (red), IL-6 (green), and nucleus (DAPI, blue) in the synovium tissue of RA patients. The scale bar is 20 μm.

sorted human $CD19^+$ B cells and cultured them under normoxia (21% O_2) or hypoxia (1% O_2) conditions. We found that the transcription of *HIF1A* increased under hypoxia (Figure 3a) and the protein levels of HIF-1α were significantly enhanced with CpG stimulation under hypoxia (Figure 3b). Correspondingly, the transcription of *IL6* was significantly upregulated when B cells were cultured under hypoxia conditions (Figure 3c). Therefore, hypoxia can induce the expression of *HIF1A* and *IL6* in human B cells.

Furthermore, CpG-activated human B cells had increased the expression level of *HIF1A* even under normoxia conditions (Supplementary figure 3a). We verified this by detecting increased HIF-1α protein levels in human B cells after anti-IgM or CpG stimulation (Supplementary figure 3b). CpG stimulation also significantly upregulated the expression of *IL6* (Supplementary figure 3c) and enhanced the IL-6 production of human B cells under both normoxia and hypoxia conditions (Figure 3d). Therefore, CpG-stimulation activates HIF-1α signalling and IL-6 expression in human B cells.

We demonstrated that Raji cells cultured under hypoxia conditions with DMOG treatment (inhibition of HIF-1α degradation) could stably produce IL-6, but KC7F2 treatment (inhibition of *HIF1A* translation) dramatically reduced the production of IL-6 (Figure 3e). Correspondingly, for human B cells sorted from peripheral blood

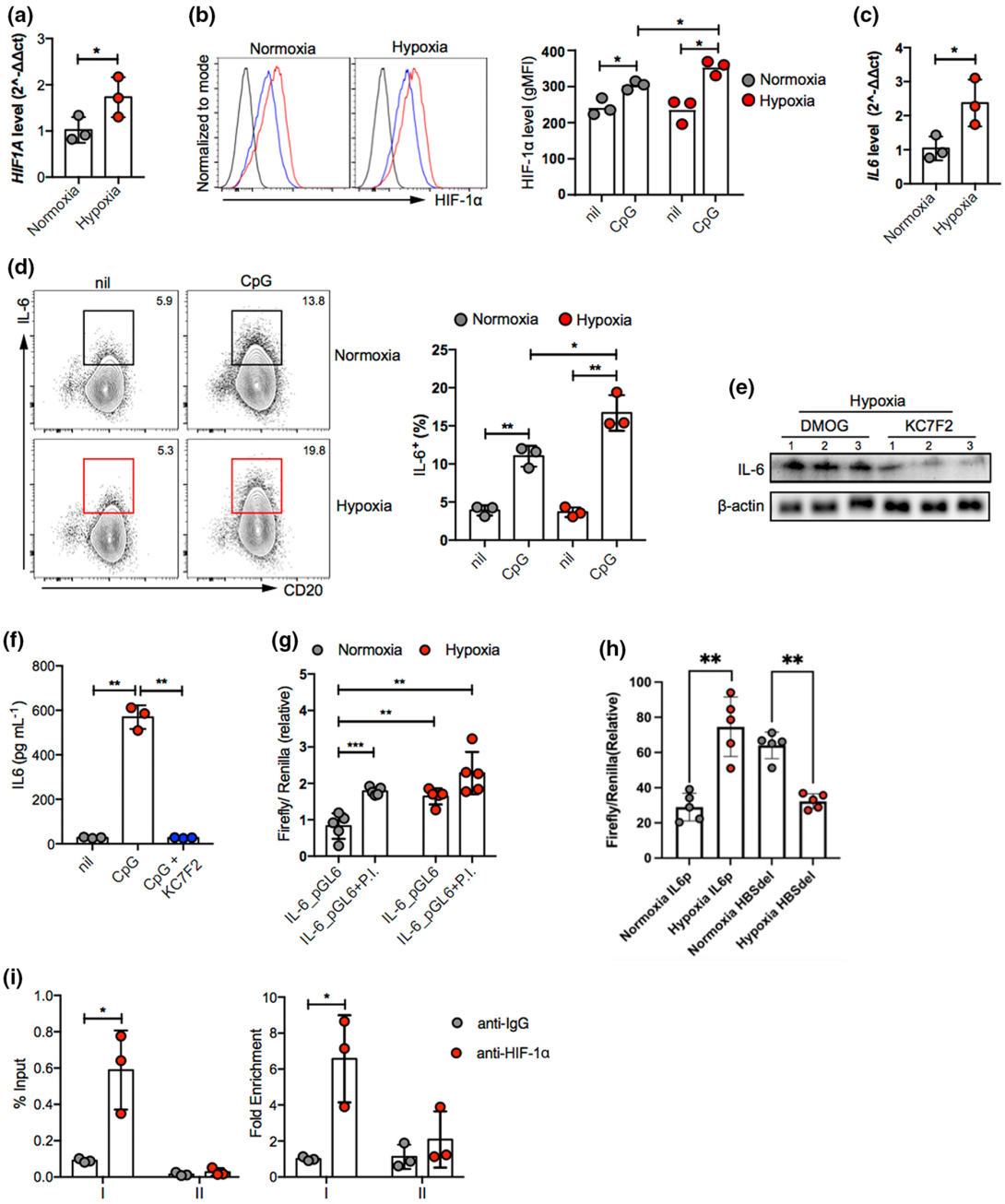

Figure 3. HIF-1α regulates IL-6 production of human B cells. **(a)** Transcript of *HIF1A* in human B cells cultured under normoxia (21% O_2) or hypoxia (1% O_2) conditions for 3 h (n = 3). **(b)** Expression of HIF-1α in human B cells culture under normoxia (21% O_2) or hypoxia (1% O_2) conditions, with or without CpG stimulation (3 μM) for 24 h (n = 3). **(c)** Transcript of *IL6* in human B cells cultured under normoxia (21% O_2) or hypoxia (1% O_2) conditions for 6 h (n = 3). **(d)** Representative plots and comparison of flow cytometric analysis of IL-6-producing human B cells under normoxia and hypoxia conditions stimulated with or without CpG (3 μM) for 24 h. **(e)** Western blot assay of IL-6 in Raji cell line cultured under hypoxia conditions for 24 h. The Raji cell line was treated by DMOG or KC7F2 (inhibitor of HIF1A transcription). **(f)** Secretion of IL-6 from human B cells stimulated by CpG (3 μM) with or without KC7F2 for 24 h. *P < 0.05, **P < 0.01. **(g)** Dual-luciferase reporter assay in 293 T cells transfected with pGL6 vector carrying *IL6* promoter region. Transfected cells were cultured under normoxia or hypoxia conditions, with or without PMA, and ionomycin treatment for 24 h. **(h)** Dual-luciferase reporter assay in B cells transfected with PGLV6-IL6-promotor and PGLV6-IL6p-HBSdel plasmids. The transfected B cells are then cultured for another 24 h under normoxia or hypoxia conditions **P < 0.01. **(i)** HIF-1α ChIP assay in human B cells treated with CpG stimulation (3 μM) under hypoxia conditions for 12 h. Results show the recruitment of HIF-1α on the predicated HER regions (I and II) of the *IL6* promoter. *P < 0.05, **P < 0.01, ***P < 0.001.

mononuclear cells (PBMCs), CpG treatment dramatically increased IL-6 secretion levels, whereas adding KC7F2 decreased the CpG-induced IL-6 levels in normoxia conditions (Figure 3f). Collectively, these results indicated that hypoxia-induced HIF-1α or stimulation-upregulated HIF-1α can accelerate IL-6 production, while the inhibition of HIF-1α reduces IL-6 production in human B cells.

Next, we constructed a vector containing the *IL6* promoter site into the PGL-6 plasmid and transfected it into 293 T cells. The transcriptional activity of the *IL6* promoter was significantly higher under hypoxia (24 h), which can stabilise HIF-1α and induce its transcriptional function (Supplementary figure 3d and e). Similarly, under normoxic conditions, phorbol 12-myristate 13-acetate (PMA) and Ionomycin stimulation led to nearly twofold upregulation of the transcriptional activity of the *IL6* promoter. Under hypoxic conditions, PMA and ionomycin stimulation further enhanced the transcriptional activity of the *IL6* promoter, which was higher than that under normoxia (Figure 3g). Therefore, the above data support that HIF-1α can bind to the promoter region of the human *IL6* gene and regulate IL-6 transcription.

To confirm this phenomenon further, we isolated human primary B cells from PBMC using flow cytometry and transfected them with PGLV6-IL6-promotor and PGLV6-IL6p-HBSdel plasmids. Dual-luciferase reporter assay demonstrated that the *IL6* promoter signal was significantly elevated under hypoxia conditions compared with normoxia conditions. Moreover, this effect was reversed when the HIF-1α-binding site was deleted from the sequence of the *IL6* promoter (Figure 3h). These results confirmed that impeding the binding of HIF-1α prevents *IL6* promoter activity.

Furthermore, we identified the consensus core sequence of hypoxia response elements (HREs) in *Homo sapiens* (*ACCTGC*) using *JASPAR* (http://jaspar.genereg.net) and found that two HREs (labelled as I and II) were present in the *IL6* promoter (Supplementary figure 3f). ChIP-PCR analysis confirmed that HIF-1α bound to the HRE-I region, which was transcriptionally activated and bound with HIF-1α after CpG stimulation under hypoxia conditions (Figure 3i).

In conclusion, HIF-1α directly regulates *IL6* transcription and promotes IL-6 production in human B cells by binding to the promoter region of the human *IL6* gene.

HIF-1α regulates IL6 expression in murine B cells *in vitro*

To confirm the role of HIF-1α in IL-6 production in murine B cells, we sorted spleen B cells from C57BL/6J mice and cultured them *in vitro*. Notably, B cells cultured under hypoxia conditions produced significantly more IL-6 than those cultured under normoxic conditions (Figure 4a). Additionally, we found that LPS-induced IL-6 production in spleen B cells was dose- and time-dependent (Supplementary figure 4a). Upon LPS stimulation, the secretion of IL-6 from cultured B cells under hypoxic conditions increased significantly more than that from B cells under normoxia (Figure 4b).

Next, we generated *Cd19-Cre: Hif1α*$^{fl/fl}$ (*Hif1α*$^{\Delta B}$) mice with HIF-1α-specific deficiency in B cells by crossing *Cd19-Cre* mice with *Hif1α*$^{flox/flox}$ mice (Figure 4c). Upon LPS stimulation, the *Il6* transcript in *Hif1α*-deficient B cells was significantly lower than that in WT (C57BL/6J mice) B cells (Figure 4d; Supplementary figure 4a and b). Consistently, *Hif1α*-deficient B cells had remarkably lower levels of IL-6 (nearly twofold reduction) after anti-IgM, CD40L or LPS treatment, either individually or in combination (Figure 4e).

Taken together, these results indicate that HIF-1α can regulate IL-6 production in murine B cells *in vitro*.

RNAseq analysis identified upregulated *HIF1A* and *IL6* in naïve B cells from patients with RA

To further confirm the relationship between HIF-1α and IL-6 in the naïve B cells of RA patients, we utilised publicly available RNAseq data (GSE110999)[29] from the GEO database and re-analysed the transcriptome of naïve B cells from RA patients ($n = 4$) and healthy individuals ($n = 3$). The 3D PCA plot demonstrates the separation of naïve B cells derived from RA and healthy controls (HC), as PC1 (88.1%) effectively distinguished between the two groups (Figure 5a). Additionally, the divergence of the RA-derived naïve B cells was greater than that of HC-derived cells, as demonstrated by PC2 and PC3. We identified upregulated 2467 genes (adjusted P-value < 0.05, Fold Change > 2) in RA-derived naïve B cells, including *IL6*, *HIF1A* and several HIF-1α-targeted genes (Figure 5b).

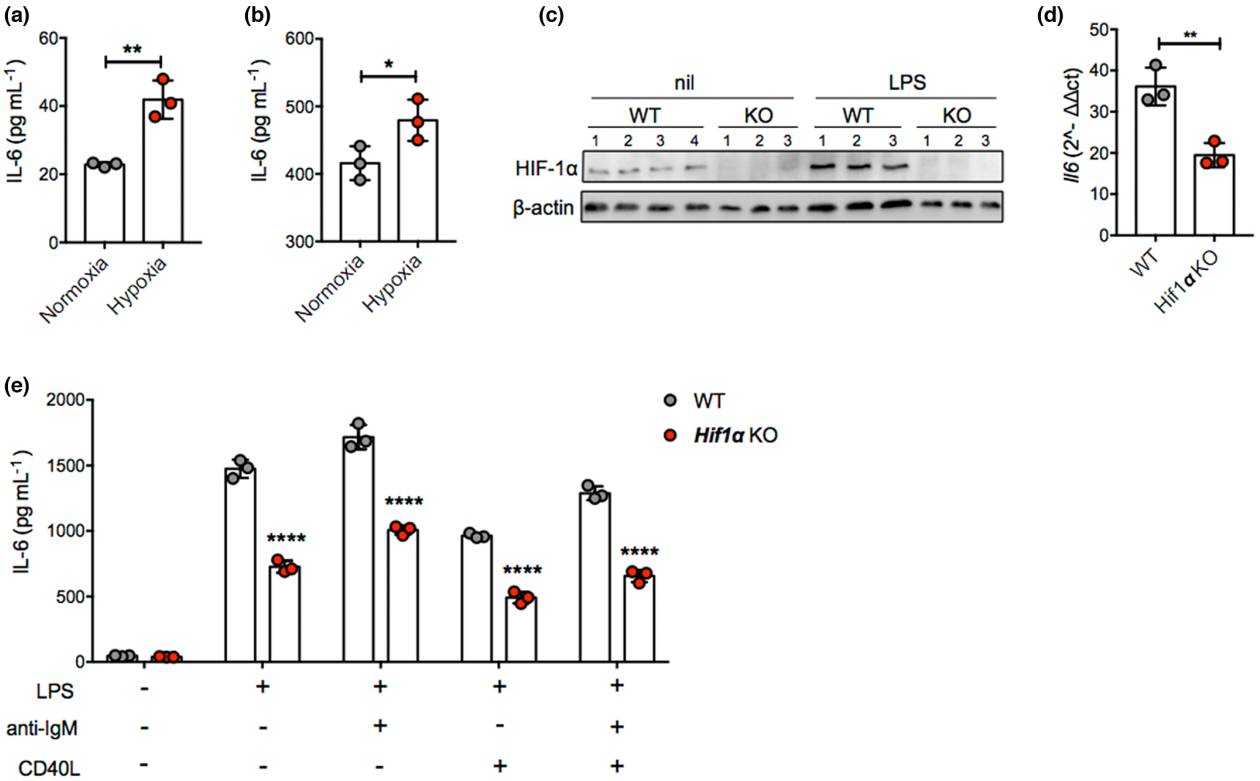

Figure 4. HIF-1α regulates IL-6 production of murine B cells *in vitro*. **(a)** Secretion of IL-6 from murine spleen B cells cultured under normoxia or hypoxia conditions for 24 h. **(b)** Secretion of IL-6 from murine spleen B cells cultured under normoxia or hypoxia conditions and stimulated with LPS (1 μg mL^{-1}) for 24 h. **(c)** Western blot analysis of HIF-1α in the splenic B cells of WT and *Hif1α*$^{\Delta B}$ mice with or without LPS (1.0 μg mL^{-1}) stimulation for 24 h. **(d)** Transcript of *Il6* in the splenic B cells of WT and *Hif1α*$^{\Delta B}$ mice. The B cells were stimulated with LPS (1.0 μg mL^{-1}) for 6 h. **(e)** Secretion of IL-6 of the splenic B cells of WT and *Hif1α*$^{\Delta B}$ mice. The B cells were stimulated with LPS (1.0 μg mL^{-1}), in the presence or absence of CD40L (1.0 μg mL^{-1}) or anti-IgM (10.0 μg mL^{-1}) for 24 h (n = 3). *P < 0.05, **P < 0.01, ****P < 0.0001.

Using gene set enrichment analysis (GSEA), we found that RA-derived naïve B cells were enriched in the *Antigen Processing and Presentation* and the *B Cell Receptor Signalling Pathway*, indicating that a subset of naïve B cells from RA patients was activated and acted as antigen-presenting cells (Figure 5c). Importantly, we also found that the upregulation of *IL6* in RA-derived naïve B cells was because of the activation of the *Toll-like Receptor Signalling Pathway* and the *NOD-like Signalling Pathway* (Figure 5c; Supplementary figure 5).

Finally, we conducted transcription factor enrichment analysis based on the upregulated genes in RA-derived naïve B cells, and HIF-1α was one of the significantly enriched transcription factors (Figure 5d).

In summary, we confirmed the upregulation of *HIF1A*, *IL6* and HIF-1α-targeted genes in the naïve B cells of RA patients.

DISCUSSION

The role of B cells in the pathogenesis of rheumatoid arthritis (RA) is well-established, but the specific mechanisms underlying their involvement are still being elucidated. In this study, we found that B cells are the major source of IL-6 in the peripheral blood of RA patients, and the ratio of IL-6-producing B cells correlated with the severity of the disease. While monocytes in circulation are known to be the primary producers of IL-6 in healthy individuals, the presence of IL-6+ monocytes in the peripheral blood of RA patients was found to be significantly reduced. Because of the reduction, the representation of naïve and nonswitched memory B cells with IL-6 capacity in circulation is increased, reflecting a disease-related shift. Furthermore, inflamed monocytes enriched in synovial tissue may differ in ability to produce IL-6 compared with circulating monocytes.[30] In

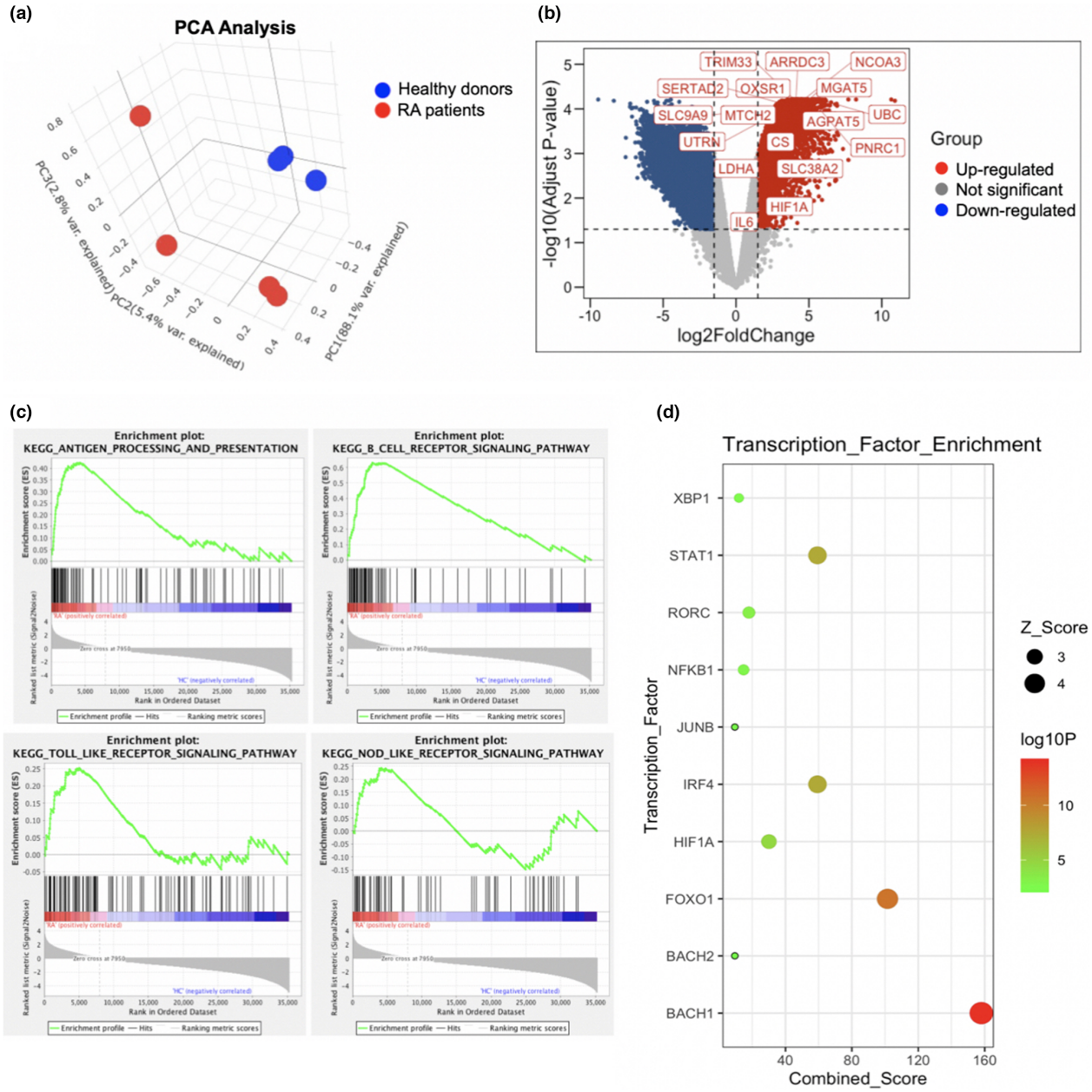

Figure 5. Naïve B cells from RA patients were metabolically activated and upregulated *HIF1A*, *IL6*, and HIF-1a targeted genes. **(a)** PCA plots based on the transcriptome of naïve B cells from RA patients (n = 4) and healthy individuals (n = 3). RNAseq data were downloaded from the GEO database (GSE110999), and re-analysed by *R*. **(b)** Volcano plots of the differentially expressed genes (adjusted *P*-value < 0.05, Fold Change > 2) between RA-derived and healthy-derived naïve B cells. Blue plots indicate the downregulated genes, while red plots represented the up-regulated genes in RA-derived naïve B cells. *HIF1A*, *IL6* and some of the HIF-1a targeted genes, such as *NCOA3*, *PNRC1*, and *CS* were upregulated in RA-derived naïve B cells. **(c)** Gene set enrichment analysis based on the transcriptome of the naïve B cells from RA patients and healthy individuals. Selected KEGG pathways that were highly enriched in RA-derived naïve B cells were shown (up-left, Antigen Processing and Presentation; up-right, the B Cell Receptor Signalling Pathway; middle-left, TCA-cycle; middle-right, Oxidative Phosphorylation; down-left, the Toll-like Receptor Signalling Pathway; down-right, the NOD-like receptor signalling pathway). **(d)** Enrichment plot of the transcription factor encoded genes that were up-regulated in RA-derived naïve B cells. The *x*-axis indicates the combined scores of each transcription factor. The size of the plots indicates the Z score of the enrichment analysis of each transcription factor. The colours of the plots indicate the transferred *P*-value ($\log_{10}$P) of the enrichment.

addition to their function of secreting antibodies[31] and presenting antigens to T cells,[32,33] B cells can produce a range of cytokines, including IL-6, TNF-α, lymphotoxin-β, as well as IL-7, IL-8 and IL-10.[12,25] Barr *et al.* were the first to report the pathogenic role of B cell-derived IL-6 in autoimmune diseases. They found that patients with multiple sclerosis (MS) had higher levels of IL-6-producing B cells than healthy controls and that B cell-specific deficiency of IL-6 could alleviate the severity of experimental autoimmune encephalomyelitis (EAE) mouse model.[34] Furthermore, B cell-derived IL-6 was shown to initiate the formation of germinal centre (GC) during the autoimmune response, and the loss of B cell-derived IL-6 blocked autoimmune GC formation, decreased production of auto-antibodies and ameliorated the disease of lupus in a mouse model.[24] However, this study is the first to identify the pathogenic involvement of B cell-derived IL-6 in RA patients. The ratio of IL-6-producing B cells significantly correlated with CRP, IgM-RFs and IgG-RFs, suggesting the pro-inflammatory effect of B cell-derived IL-6 and its role in immunoglobulin class-switch and auto-antibody production.

We have identified naïve B cells as the main subset of B cell-producing IL-6 in the peripheral blood of RA patients. Previous research by Glaum *et al*. in 2009 showed that $CD19^{+}CD27^{-}$ human naïve B cells could secrete IL-6,[35] but did not compare productivity between different B cell subsets. In addition, circulating plasmablasts were suggested to produce IL-6 and induce Tfh differentiation.[36] While in mice, $CD21^{hi}CD23^{low}$ marginal zone B cells and $CD1d^{hi}$ B cells were reported to have a greater capacity to secrete IL-6.[34,37] This study fills an important gap in the literature by using multiple approaches to demonstrate that naïve B cells are the main IL-6-producing B cell subset in RA patients. Zhang *et al.* combined scRNAseq and mass cytometry to investigate the production of IL-6 in synovial tissue of RA patients. They found that the majority of $IL\text{-}6^{+}$ cells were $HLA\text{-}DRA^{hi}$ fibroblasts and naïve B cells, which is consistent with our findings.[3] Additionally, synovial-infiltrating monocytes were found to be the primary producers of IL-1β.

After examining the transcriptional characteristics of naive B cells, we discovered a relationship between IL6 and STAT6, HIF1A, and MAPK1. The transcription of the Hif1a gene can be regulated by the NF-kB or IL6-IL6R-JAK-STAT3 signalling pathways.[38,39] A previous study has shown an increase in IL-6 levels in RA patients.[6] In this study, we observed the enrichment of the Toll-like receptor signalling pathway (Figure 5c) in B cells from RA patients, which would activate NF-kB. Together, these findings suggest that Hif1a gene transcription is increased in B cells of RA patients. Using flow cytometry and immunofluorescence, we found that HIF-1α and IL-6 were co-expressed in peripheral B cells and synovial B cells of RA patients. The enrichment of B cell cytoplasmic HIF-1α suggests elevated transcription and accumulation, or reduced degradation, which is related to the pro-inflammatory microenvironment and hypoxic conditions in RA synovial tissue. Since only a small fraction of the HIF-1α complex will transport into the nucleus when performing its function, and its mobility in the nucleus,[40,41] detecting intranuclear HIF-1α, especially in formalin-fixed tissue, is challenging.

We hypothesised that HIF-1α might regulate IL-6 production by B cells. Gao *et al*.[14] reported a link between HIF-1α and STAT3 in regulating IL-6 production in the synovium of RA patients. In our study, we focussed on B cells and cultured them under hypoxia conditions to mimic the pathogenic hypoxia status of RA synovium. Under hypoxia, HIF-1α and IL-6 increased in both human and murine B cells. The immune cell activation, such as TCR-PI3K/mTOR signalling pathway activation in T cells[42] and Toll-like receptor (TLR)-nuclear factor κB (NF-κB) signalling pathway activation in monocytes and macrophages,[43,44] has been proved to promote the protein synthesis of HIF-1α. Similarly, we found the CpG- or LPS stimulation, which induced B cell activation, can also increase the production of HIF-1α and IL-6. Mechanistically, we demonstrated that hypoxia and/or B cell activation can enhance HIF-1α binding to the *IL6* promoter and regulate *IL6* gene transcription.

Controversially, Meng *et al*.[11] reported that HIF-1α regulates B cells to secrete IL-10 and that the loss of IL-10-producing $CD1d^{hi}CD5^{+}$ B1 cells in $Mb1^{cre}Hif1\alpha^{f/f}$ mice leads to an aggravation of arthritis. Since the differentiation of IL-10-producing regulatory B cells is induced by IL-1β and IL-6,[45] it is possible that the reduced IL-6 production of B cells consequently dampens IL-10 production in $Mb1^{cre}Hif1\alpha^{f/f}$ mice. However, our results show that HIF-1α-dependent IL-10 production is limited to regulatory B cells, while HIF-1α-dependent IL-6 expression is mainly in

effective B cells. The roles of HIF-1α on IL-6 and IL-10 production in B cells are complicated and require further investigation.

In summary, we revealed in this study that B lymphocytes are the primary source of IL-6 in the peripheral blood of individuals with RA and that naïve B cells are the main subset of B cell-producing IL-6. Mechanistically, we observed that HIF-1α regulates IL-6 production in B cells by directly targeting the gene promoter region to activate the transcription. Taken together, our findings suggest that targeting HIF-1α may provide a new therapeutic option in RA.

METHODS

Patients

Thirty-four patients diagnosed with RA who fulfilled the 2010 classification criteria of the American College of Rheumatology/European League Against Rheumatism were recruited. None of the patients had received the biologic agents or high-dose corticosteroids (> 10 mg per day) treatment within 6 months prior to the study. We also recruited 42 healthy controls with comparable demographics. The study was approved by the Ethical Committee of Renji Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). All participants were informed with written consent forms. The demographic characteristics of the healthy controls and patients with RA are shown in Table 1.

Table 1. Clinical characteristics of RA patients and healthy donors

Characteristics	RA (n = 34)	HD (n = 42)
Age, mean (range), years	54 (28–74)	44 (27–67)
Sex, female (%)	30 (88.2)	33 (78.6)
Alanine aminotransferase, U L^{-1}	25.7 (6–81)	18 (7–49)
Creatinine, μmol L^{-1}	54.7 (29–75)	61.8 (37–93.2)
White blood count, 10^9 L^{-1}	7.15 (4.05–18.78)	5.5 (3.54–8.94)
Proportion of neutrophils, %	65.2 (45.9–83.3)	59.9 (41.3–78.3)
Proportion of lymphocyte, %	26.1 (12–41.8)	32 (16.9–50.6)
Haemoglobin, g L^{-1}	130 (113–149)	136.8 (95–177)
Blood platelet, 10^9 L^{-1}	269.5 (138–415)	257.8 (162–394)
Duration, mean (range), years	3.23 (0–30)	
Tender joint count, median (range) of 28 joints	8.29 (0–23)	
Swollen joint count, median (range) of 28 joints	7.59 (0–23)	
DAS28, mean (range)	5.34 (1.92–7.83)	
ESR, mean (range), mm h^{-1}	39.35 (4–95)	
CRP, mean (range), mg L^{-1}	16.9 (< 3.13–135.4)	
RF, mean (range), IU mL^{-1}	278.19 (< 9.18–1220)	
Anti-CCP antibody, mean (range), U mL^{-1}	5.38 (0.2–12.4)	
Anti-rheumatic therapy (%)	16 (47.1%)	

anti-CCP antibody, anticyclic citrullinated peptide antibody; CRP, C-reactive protein; DAS28, disease activitz score-28; ESR, erythrocyte sedimentation rate; HD, healthy donors; RA, rheumatoid arthritis (patients); RF, rheumatoid factor.

Mice

WT C57BL/6J mice, *Cd19-Cre* mice and *Hif1a*$^{flox/flox}$ mice were purchased from the Jackson Laboratory and maintained under specific pathogen-free conditions in the animal facility of Renji Hospital, Shanghai Jiao Tong University School of Medicine, China. All mice experiments were performed according to the animal welfare guidelines under approved protocols of Renji Hospital, School of Medicine, Shanghai Jiao Tong University, China.

Peripheral blood mononuclear cells isolation

Plasma samples were collected and stored at −80°C and PBMC were isolated with Ficoll-Paque (GE Healthcare, Chicago, USA).

Cell culture

B cells from PBMCs were isolated with anti-human CD19 MACS beads (Miltenyi Biotech, San Diego, USA). B cells (purity > 95%) were then cultured in RPMI 1640 with 10% FBS. 1 μg mL^{-1} or 3 μM CpG ODN 2006 (InvivoGen, San Diego, USA), 10 μg mL^{-1} anti-human IgM F(ab')2 Abs (Jackson ImmunoResearch, West Grove, USA), 2-Deoxy-D-glucose (5 mM; Millipore Sigma, Darmstadt, Germany), or Rotenone plus Antimycin (0.5 μM; Millipore Sigma) were used to stimulate the human B cells under normoxia (21% O_2) or hypoxia (1% O_2) for 6–72 h.

Mice splenic B cells were isolated by CD45R/B220 MACS beads (Miltenyi Biotech), and cultured in RPMI 1640 with 10% FBS. For the cultured B cells, 0.1–10 μg mL^{-1} LPS (Sigma-Aldrich, St. Louis, MO, USA), 5–10 μg mL^{-1} anti-mouse IgM F (ab') 2 Abs (Jackson ImmunoResearch) or 1 μg mL^{-1} mouse CD40L (eBioscience, San Diego, CA, USA) were added under normoxia (21% O_2) or hypoxia (1% O_2) conditions for 6–72 h.

Flow cytometry

Peripheral blood mononuclear cells were stained with fluorochrome-conjugated antibodies: CD19 (clone: 4G7),

CD3 (clone: HIT3A), CD4 (clone: A161A1), CD8 (clone: SK1), CD14 (clone: 63D3), CD16 (clone: 3G8), CD56 (clone: 5.1H11), HLA-DR (clone: L243), CD20 (clone: 2H7), CD24 (clone: ML5), CD27 (clone: M-T271), CD38 (clone: HB-7), IgD (clone: IA6-2), CD11c (clone: 3.9), IL-6 (clone: MQ2-13A5) and HIF-1α (clone: 546–16), IgG2aκ isotype control (clone: RMG2b-1) were obtained from BD Pharmagen (San Diego, CA, USA) or Biolegend (San Diego, CA, USA). Live/dead cells were distinguished using Zombie NIR Fixable Viability kit (Biolegend). Data were collected by a flow cytometer (Fortessa X20, BD) and analysed by the FlowJo software (BD).

Cell sorting and transfection

Peripheral blood mononuclear cells were isolated from two healthy donors' whole venous blood using GE Ficoll-Paque through a density gradient centrifugation protocol. Then, human PBMCs were stained by PE-CF594 linked anti-CD19 antibody and Zombie Aqua Fixable Dye for 20 min before sorting. Flow cytometry sorting assay was conducted using a BD Aria III FCM. 200 000 B cells were sorted from each sample and collected in PBS in a 5-mL Falcon test tube and centrifuged 450 *g* for 5 min at room temperature. The supernatant was removed and cells are resuspended in 10%FBS IMDM medium. B cells were then partitioned into two 96-well cell-culturing plates for 50 000 cells per well. Sorted cells were next cultured in 5%CO_2 37°C incubator for 24 h. This incubation period allowed the fluorescence of the cells to become fully quenched.

ELISA

Human IL-6 levels in the serum and the supernatant of cultured B cells were measured using an anti-human IL-6 ELISA kit (Biolegend). IL-6 production of cultured B cells was measured using an anti-mouse IL-6 ELISA kit (R&D, Minneapolis, MN, USA) following the manufacturer's protocol.

Immunoblot

Raji cells stimulated with DMOG or KF7C2, under normoxia or hypoxia, WT and *Hif1a*–deficient mouse B cells stimulated with LPS, and human B cells stimulated by IgM or CpG were lysed using RIPA (Sigma-Aldrich) with protease inhibitor complete (Roche, Mannheim). Primary antibodies, including anti-human IL-6 antibody (poly clone, Abcam, Cambridge, UK), HIF-1α antibody (clone: mgc3, Abcam) and β-actin (13E5, Cell Signaling Technology, Danvers, MA, USA) Rabbit mAb were used. Gel bands were analysed using the Photoshop CS4 software (Adobe).

Immunofluorescence staining

Synovium was collected, fixed, processed and stained with anti-human CD19 antibody (clone: EPR5906, Abcam), HIF-1α antibody (clone: mgc3, Abcam) and IL-6 antibody (poly clone, Abcam), and nucleus was stained with DAPI. Images were captured with a laser scanning confocal microscope (Zeiss) and analysed using the Photoshop CS4 software (Adobe).

Quantitative real-time PCR

Total RNA was extracted with TRIzol™ Reagent (Life Technologies, Carlsbad, CA, USA), and cDNA was synthesised with a reserve transcription kit (TOYOBO, Japan). The mRNA level of the target gene was measured by real-time PCR (ABI 7900 System) using the SYBR Green Master Mix (Takara, Japan) with primers in Supplementary table 1. The relative expression level of target genes was calculated by normalising to β-actin values using the $2^{(-\Delta\Delta CT)}$ method.

Luciferase reporter assay

The human IL6 promoter region (Supplementary table 2) was cloned into the pGL6 firefly reporter vector. The vector was transfected into 293 T cells using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and cultured under normoxia (21% O_2) or hypoxia (1% O_2) conditions for 24 h. Cells were collected, lysed, and luciferase activity was measured using Dual-Glo® Luciferase Assay System (Promega, Madison, WI, USA) in a Centro LB 960 Microplate Luminometer (Berthold).

Human B cells are transfected with PGLV6-IL6-promotor and PGLV6-IL6p-HBSdel plasmids using Thermo Fishers' Lipofectamine 3000 cell transfecting kit. The transfected B cells were then cultured for another 24 h in a 5% CO_2 37°C incubator, with one plate placed in an Anaeropack to create a hypoxia situation while the other was placed in a normoxia environment before protein harvesting. Transfected human B cells were centrifuged at 4°C for 5 min at 450 *g* and then resuspended in RIPA lysis. After incubating on ice for 30 min, cell culturing plates were centrifuged at 4°C for 5 min at 1000 *g*. Cell lysis was transferred to a new black 96-well plate, with 20 μL per well. The dual-luciferase reporter assay was performed using Promega's dual-luciferase reporting kit. A volume of 100 μL LAR was added to cell lysis for the first fluorescent signal measurement and 100 μL Stop&Glo reagent was added for the second measurement.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed using the EZ-ChIP™-Chromatin Immunoprecipitation Kit (Merk Millipore, Burlington), following the manufacturer's instructions. Briefly, 1×10^7 human $CD19^+$ B cells were treated with CpG (1 μg mL^{-1}) under hypoxia conditions for 12 h. Immunoprecipitation was performed using anti-HIF-1α (clone: BL-124-3F7), anti-IgG (negative control) and anti-RNA Polymerase (positive control) antibodies. The human HIF-1α binding sites (ACGTGC) in the promoter region of human IL6 were predicated by JASPAR. DNA was purified and analysed by quantitative real-time PCR (ABI 7900) using primers targeting the *IL6* promoter (Supplementary table 3).

Online data download and analysis

We searched the GEO database (https://www.ncbi.nlm.nih.gov/geo/) and downloaded GSE42724 and GSE110999. The data were re-analysed using R (version 3.6.3). Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway enrichment analysis of the upregulated genes were performed by R Packages *clusterProfiler*, *enrichplot* and *ggplot2*. The significantly activated biological processes, molecular functions, cellular components and signalling pathways were explored. *STRING* tool (https://string-db.org/) was used to construct the Protein–Protein Interaction (PPI) network of the identified genes (CI = 0.90). The network was reconstructed using *CytoHubba*, a plug-in of Cytoscape software (version 3.5). Gene set enrichment analysis for KEGG pathway was conducted using GSEA tool (https://www.gsea-msigdb.org/gsea/index.jsp). $P < 0.05$ was considered statistically significant.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 7.0, GraphPad Software) or R (version 3.6.2). The Mann–Whitney *t*-test and one-way ANOVA or two-way ANOVA were used to compare nonparametric and parametric data, respectively. Spearman's correlation analysis was used to test the relationship between the two factors. $P < 0.05$ was considered to be statistically significant.

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AUTHOR CONTRIBUTIONS

Chaofan Fan: Conceptualization; data curation; formal analysis; methodology; project administration; software; validation; visualization; writing – original draft. **Jia Li:** Formal analysis; investigation; project administration; writing – original draft; writing – review and editing. **Yixuan Li:** Data curation; formal analysis; methodology; project administration. **Yuyang Jin:** Data curation; formal analysis; investigation; methodology. **Jiaqi Feng:** Data curation; formal analysis; investigation; methodology. **Ruru Guo:** Data curation; resources. **Xinyu Meng:** Data curation; resources. **Dongcheng Gong:** Data curation; resources. **Qian Chen:** Data curation; software. **Fang Du:** Funding acquisition; resources. **Chunyan Zhang:** Data curation; resources. **Liangjing Lu:** Conceptualization; funding acquisition; project administration; supervision. **Jun Deng:** Conceptualization; formal analysis; funding acquisition; methodology; supervision; writing – review and editing. **Xiaoxiang Chen:** Conceptualization; funding acquisition; investigation; methodology; supervision; writing – review and editing.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

REFERENCES

1. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet* 2016; **388**: 2023–2038.
2. Malmstrom V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *Nat Rev Immunol* 2017; **17**: 60–75.
3. Zhang F, Wei K, Slowikowski K *et al*. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol* 2019; **20**: 928–942.
4. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; **423**: 356–361.
5. van Hamburg JP, Tas SW. Molecular mechanisms underpinning T helper 17 cell heterogeneity and functions in rheumatoid arthritis. *J Autoimmun* 2018; **87**: 69–81.
6. Deng J, Fan C, Gao X *et al*. Signal transducer and activator of transcription 3 hyperactivation associates with follicular helper T cell differentiation and disease activity in rheumatoid arthritis. *Front Immunol* 2018; **9**: 1226.
7. Xiao XY, Li YT, Jiang X *et al*. EZH2 deficiency attenuates Treg differentiation in rheumatoid arthritis. *J Autoimmun* 2020; **108**: 102404.
8. Takeshita M, Suzuki K, Kondo Y *et al*. Multi-dimensional analysis identified rheumatoid arthritis-driving pathway in human T cell. *Ann Rheum Dis* 2019; **78**: 1346–1356.
9. Yeo L, Lom H, Juarez M *et al*. Expression of FcRL4 defines a pro-inflammatory, RANKL-producing B cell subset in rheumatoid arthritis. *Ann Rheum Dis* 2015; **74**: 928–935.
10. Yang M, Deng J, Liu Y *et al*. IL-10-producing regulatory B10 cells ameliorate collagen-induced arthritis via suppressing Th17 cell generation. *Am J Pathol* 2012; **180**: 2375–2385.
11. Meng X, Grotsch B, Luo Y *et al*. Hypoxia-inducible factor-1alpha is a critical transcription factor for IL-10-producing B cells in autoimmune disease. *Nat Commun* 2018; **9**: 251.
12. Kristyanto H, Blomberg NJ, Slot LM *et al*. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. *Sci Transl Med* 2020; **12**: eaaz5327.
13. Ng CT, Biniecka M, Kennedy A *et al*. Synovial tissue hypoxia and inflammation in vivo. *Ann Rheum Dis* 2010; **69**: 1389–1395.
14. Gao W, McCormick J, Connolly M, Balogh E, Veale DJ, Fearon U. Hypoxia and STAT3 signalling interactions regulate pro-inflammatory pathways in rheumatoid arthritis. *Ann Rheum Dis* 2015; **74**: 1275–1283.

15. Semenza GL. Oxygen sensing, homeostasis, and disease. *N Engl J Med* 2011; **365**: 537–547.
16. Haschemi A, Kosma P, Gille L *et al*. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab* 2012; **15**: 813–826.
17. Dang EV, Barbi J, Yang HY *et al*. Control of T_H17/T_{reg} balance by hypoxia-inducible factor 1. *Cell* 2011; **146**: 772–784.
18. Zhu Y, Zhao Y, Zou L, Zhang D, Aki D, Liu YC. The E3 ligase VHL promotes follicular helper T cell differentiation via glycolytic-epigenetic control. *J Exp Med* 2019; **216**: 1664–1681.
19. Kojima H, Gu H, Nomura S *et al*. Abnormal B lymphocyte development and autoimmunity in hypoxia-inducible factor 1alpha-deficient chimeric mice. *Proc Natl Acad Sci USA* 2002; **99**: 2170–2174.
20. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 2015; **16**: 448–457.
21. Niu Q, Huang ZC, Wu XJ *et al*. Enhanced IL-6/phosphorylated STAT3 signaling is related to the imbalance of circulating T follicular helper/T follicular regulatory cells in patients with rheumatoid arthritis. *Arthritis Res Ther* 2018; **20**: 200.
22. Gottenberg JE, Dayer JM, Lukas C *et al*. Serum IL-6 and IL-21 are associated with markers of B cell activation and structural progression in early rheumatoid arthritis: results from the ESPOIR cohort. *Ann Rheum Dis* 2012; **71**: 1243–1248.
23. Komatsu N, Okamoto K, Sawa S *et al*. Pathogenic conversion of $Foxp3^+$ T cells into TH17 cells in autoimmune arthritis. *Nat Med* 2014; **20**: 62–68.
24. Arkatkar T, Du SW, Jacobs HM *et al*. B cell-derived IL-6 initiates spontaneous germinal center formation during systemic autoimmunity. *J Exp Med* 2017; **214**: 3207–3217.
25. Marston B, Palanichamy A, Anolik JH. B cells in the pathogenesis and treatment of rheumatoid arthritis. *Curr Opin Rheumatol* 2010; **22**: 307–315.
26. Covens K, Verbinnen B, Geukens N *et al*. Characterization of proposed human B-1 cells reveals pre-plasmablast phenotype. *Blood* 2013; **121**: 5176–5183.
27. Konisti S, Kiriakidis S, Paleolog EM. Hypoxia—a key regulator of angiogenesis and inflammation in rheumatoid arthritis. *Nat Rev Rheumatol* 2012; **8**: 153–162.
28. Palazon A, Goldrath AW, Nizet V, Johnson RS. HIF transcription factors, inflammation, and immunity. *Immunity* 2014; **41**: 518–528.
29. Wang S, Wang J, Kumar V *et al*. IL-21 drives expansion and plasma cell differentiation of autoreactive $CD11c^{hi}$T-bet^+ B cells in SLE. *Nat Commun* 2018; **9**: 1758.
30. Swirski FK, Nahrendorf M, Etzrodt M *et al*. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* 2009; **325**: 612–616.
31. Kuhn KA, Kulik L, Tomooka B *et al*. Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. *J Clin Invest* 2006; **116**: 961–973.
32. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. *J Immunol* 2001; **167**: 4710–4718.
33. Lund FE, Randall TD. Effector and regulatory B cells: modulators of $CD4^+$ T cell immunity. *Nat Rev Immunol* 2010; **10**: 236–247.
34. Barr TA, Shen P, Brown S *et al*. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J Exp Med* 2012; **209**: 1001–1010.
35. Glaum MC, Narula S, Song D *et al*. Toll-like receptor 7-induced naive human B-cell differentiation and immunoglobulin production. *J Allergy Clin Immunol* 2009; **123**: 224–230.e224.
36. Chavele KM, Merry E, Ehrenstein MR. Cutting edge: circulating plasmablasts induce the differentiation of human T follicular helper cells via IL-6 production. *J Immunol* 2015; **194**: 2482–2485.
37. Honda S, Sato K, Totsuka N *et al*. Marginal zone B cells exacerbate endotoxic shock via interleukin-6 secretion induced by Fcalpha/muR-coupled TLR4 signalling. *Nat Commun* 2016; **7**: 11498.
38. Niu G, Briggs J, Deng J *et al*. Signal transducer and activator of transcription 3 is required for hypoxia-inducible factor-1alpha RNA expression in both tumor cells and tumor-associated myeloid cells. *Mol Cancer Res* 2008; **6**: 1099–1105.
39. D'Ignazio L, Bandarra D, Rocha S. NF-κB and HIF crosstalk in immune responses. *FEBS J* 2016; **283**: 413–424.
40. Taylor SE, Bagnall J, Mason D, Levy R, Fernig DG, See V. Differential sub-nuclear distribution of hypoxia-inducible factors (HIF)-1 and −2 alpha impacts on their stability and mobility. *Open Biol* 2016; **6**: 160195.
41. Hu J, Bernardini A, Fandrey J. Optical analysis of hypoxia inducible factor (HIF)-1 complex assembly: imaging of cellular oxygen sensing. *Adv Exp Med Biol* 2016; **903**: 247–258.
42. Nakamura H, Makino Y, Okamoto K *et al*. TCR engagement increases hypoxia-inducible factor-1 alpha protein synthesis via rapamycin-sensitive pathway under hypoxic conditions in human peripheral T cells. *J Immunol* 2005; **174**: 7592–7599.
43. Frede S, Stockmann C, Freitag P, Fandrey J. Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-kappaB. *Biochem J* 2006; **396**: 517–527.
44. Rius J, Guma M, Schachtrup C *et al*. NF-κB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature* 2008; **453**: 807–811.
45. Rosser EC, Oleinika K, Tonon S *et al*. Regulatory B cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. *Nat Med* 2014; **20**: 1334–1339.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Graphical Abstract

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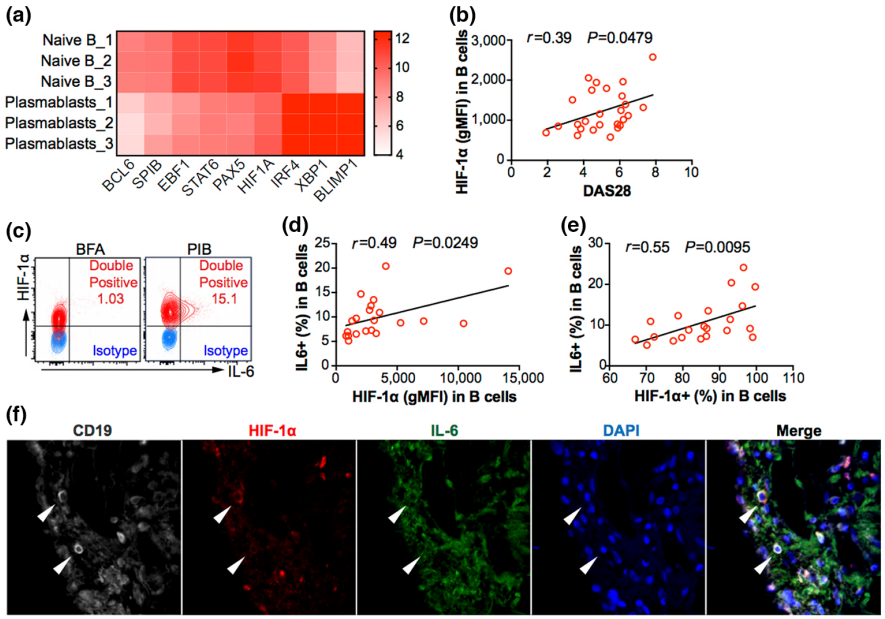

This study reveals that the CD27^{-}IgD^{+} naive B cell subset is the predominant source of IL-6 production in the peripheral blood of rheumatoid arthritis (RA) patients. Moreover, the proportion of IL-6-producing B cells is significantly correlated with the level of RA disease activity. Our findings indicate that hypoxia-inducible factor 1a directly binds to the IL6 promoter and augments its transcription.