Published in final edited form as:
Eur J Immunol. 2012 May 1; 42(5): 1226–1236. doi:10.1002/eji.201142053.

Influence of hypoxia-inducible factor 1α on dendritic cell differentiation and migration

Theresa Köhler[1], **Boris Reizis**[2], **Randall S. Johnson**[3], **Heike Weighardt**[1], and **Irmgard Förster**[1]

[1]Molecular Immunology, IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

[2]Department of Microbiology & Immunology, Columbia University, New York, USA

[3]Department of Physiology Development & Neuroscience, University of Cambridge, Cambridge, UK

Abstract

Dendritic cells (DCs) are important sentinels of the immune system and frequently reside in areas of low oxygen availability, in particular in the course of inflammatory processes. Hypoxia-inducible transcription factor (HIF)1α is responsible for major alterations in gene expression as part of the cellular adaptation to low oxygen concentration. In this study, we generated mice with a conditional deletion of HIF1α in DCs. Bone marrow-derived DCs from WT and conditional mutant mice expressed elevated levels of major histocompatibility complex class II and CD86 when grown in a hypoxic environment, whereas production of the cytokines interleukin (IL)-12p70, IL-10, IL-6, TNF-α, IL-1β, and IL-23 was reduced, both independent of HIF1α expression. In contrast, secretion of IL-22 was strongly enhanced under hypoxic conditions in an HIF1α-dependent manner. The chemokine receptor CCR7 was expressed at higher levels in wild-type DCs compared with HIF1α-deficient DCs, whereas the production of CCL17 and CCL22 was increased in conditions of low oxygen. Using in vitro as well as in vivo migration assays, we observed an enhanced migratory capability of DCs generated under hypoxia, which was HIF1α-dependent. Taken together, our data indicate that HIF1α plays an important role for DC differentiation and migration in a low oxygen environment.

Keywords

CCL17; Chemotaxis; Cre recombinase; HIF1α; IL-22

Introduction

The transcription factor hypoxia-inducible factor (HIF)1α is essential for the survival of cells under low oxygen conditions and controls cell adaptation to hypoxia. Among many

***Full correspondence*:** Dr. Irmgard Förster, Molecular Immunology, IUF–Leibniz Research Institute for Environmental Medicine, Auf'm Hennekamp 50, D-40225 Düsseldorf, Germany, Fax: +49 211 312976, Irmgard.Foerster@uni-duesseldorf.de.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

Europe PMC Funders Author Manuscripts

other changes in gene expression, this adaptation includes the conversion from oxidative phosphorylation to anaerobic glycolysis [1]. Under normoxic conditions, HIF1α is expressed constitutively, hydroxylated by prolyl hydroxylases (PHD1, PHD2, and PHD3), and degraded in a ubiquitin-dependent manner by the proteasome. Hypoxia stabilizes HIF1α by inhibition of the prolyl hydroxylases. HIF1α then accumulates and translocates into the nucleus, where it binds to HIF1β. The HIF1α/HIF1β heterodimer binds to hypoxia-responsive elements (HREs) located in promoters of a variety of genes involved in metabolism, angiogenesis, cell–cell interactions, apoptosis, and migration [2, 3], and induces the production of antimicrobial peptides such as elastases and cathepsins [4]. Furthermore, HIF1α is indispensable for cardiovascular development and HIF1α$^{-/-}$ mice survive no longer than embryonic day 11 [5]. Conditional deletion of HIF1α in T lymphocytes resulted in cytokine dysregulation and alterations in the differentiation of regulatory T cells [6–8]. Ablation of HIF1α in macrophages revealed that immigration of phagocytes into sites of inflammation and the production of antimicrobial effector molecules, such as defensins, proteases, and nitric oxide, critically depend on HIF1α [9].

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) at the interface between innate and adaptive immunity [10–12]. One of the essential functions of DCs is to migrate into sites of inflammation where they may be located in a low oxygen environment. After Ag uptake and maturation, they emigrate from the inflamed tissue to the draining lymph nodes (LNs). En route, DCs are guided by complex chemokine gradients and therefore need to orchestrate the expression of relevant chemokine receptors, as well as the production of chemokines themselves [13]. This process may also be influenced by hypoxia, as low oxygen tension significantly influences migration and the chemokine expression profile of immune cells [14–17]. To directly study the role of HIF1α in DCs, we crossed HIF1α^{flox} mice [9] with two different mouse strains expressing the Cre-recombinase in the DC lineage. CD11cCre mice have previously been shown to efficiently delete loxP-flanked target genes in the vast majority of DCs [18]. We also generated a new Cre-expressing mouse line (CCL17Cre) in which Cre is expressed under control of the *ccl17* promoter. As shown previously by our group, the inflammatory chemokine CCL17 can be induced by toll-like receptor (TLR) stimulation and is expressed by a subset of CD11b^{+} myeloid DCs that is primarily found in LNs and peripheral organs, such as skin and gut [19, 20]. These tissues are known to contain low concentrations of oxygen [21, 22]. In the following, we have examined the functional properties of bone marrow derived DCs (BMDCs) derived from mice with a cell-type specific deletion of HIF1α.

Results

Conditional deletion of HIF1α in DCs

To explore the function of HIF1α in DCs, we created two independent mouse strains with a conditional deletion of HIF1α in DCs by crossing HIF1α^{flox} mice [9] with either CD11cCre or CCL17Cre mice. CCL17Cre mice were generated by insertion of a cDNA encoding Cre into the second exon of *ccl17* (Fig. 1A). To get insight into the efficiency and specificity of Cre-mediated deletion in CD11cCre × HIF1α^{flox} (cHIF1α^{CD11c}) and CCL17Cre × HIF1α^{flox} (cHIF1α^{CCL17}) mice, we performed Southern blot analyses of BMDCs. The

deletion efficiency was calculated by comparison of the density of wild-type (WT) and recombined bands. BMDC cultures contained approximately 65% CD11c-positive cells. Around 50% of these expressed CCL17. In line with this expectation, Southern blotting revealed deletion of HIF1α in 40% of the cells in cHIF1α^{CCL17} BM cultures and more than 70% deletion of HIF1α in BM cultures from cHIF1α^{CD11c} mice (Fig. 1B). However, analysis of purified CCL17- or CD11c-positive DCs from cHIF1α^{CCL17} or cHIF1α^{CD11c} mice, respectively, showed a deletion efficiency of more than 90%. The recombined 1.5 kb band was not detected in HIF1α^{flox} cells in the absence of a Cre transgene. These data show that Cre-mediated deletion in BMDCs of either cHIF1α^{CD11c} or cHIF1α^{CCL17} is highly efficient, considering that CCL17 is mainly expressed in mature but not immature myeloid DCs [19].

Certain subsets of activated T cells are known to also express CD11c [23]. We detected deletion of the HIF1α gene in a substantial proportion of CD3$^+$ T lymphocytes in the spleen of cHIF1α^{CD11c} mice, that is, 30% in one (Fig. 1B) and 70% in another experiment (data not shown). Cre-mediated deletion affected both CD4$^+$ and CD8$^+$ T cells as additionally shown by breeding of the CD11cCre line to an enhanced green fluorescent protein (EGFP) reporter line (RA/EG mice [24]) (Supporting Information Fig. 1). In contrast, no Cre-mediated deletion was detected in splenic T cells of cHIF1α^{CCL17} and HIF1α^{flox} mouse lines (Fig. 1B). We conclude from this analysis that both conditional knockout mouse lines are suitable for analysis of HIF1α function in BMDCs, with near complete deletion in cHIF1α^{CD11c} BMDCs and a more restricted deletion in cHIF1α^{CCL17} BMDCs. For analysis of HIF1α function in vivo, use of cHIF1α^{CD11c} mice will affect both DCs and a substantial proportion of T cells, whereas T cells are not affected in cHIF1α^{CCL17} mice.

Hypoxia leads to reduced cell growth but enhanced maturation of BMDCs

To analyze the impact of hypoxia on the differentiation and maturation of DCs, we generated BMDCs from HIF1α^{flox}, cHIF1α^{CD11c}, and cHIF1α^{CCL17} mice under normoxic (21% O_2) or hypoxic (1% O_2) conditions. BMDCs differentiated under hypoxia showed reduced growth compared with BMDCs differentiated under normoxic conditions, as determined by the total cell recovery at day 6 of the BM cell culture. In addition, HIF1α-deficient BMDCs of cHIF1α^{CD11c} and cHIF1α^{CCL17} mice revealed further growth retardation compared with HIF1α^{flox} controls under hypoxic conditions. Slight differences were also seen in the frequency of CD11c$^+$ cells in the different types of BM culture (Fig. 2A). A possible explanation for the reduced growth and the reduced percentage of CD11c$^+$ cells in HIF1α-deficient BMDCs under hypoxia may be a defect in energy metabolism in the absence of HIF1α, as previously shown for HIF1α-deficient macrophages [9]. In line with this, reduced amounts of ATP were found in cell lysates of cHIF1α^{CD11c} BMDCs in contrast to HIF1α^{flox} BMDCs under hypoxia (Fig. 2B). In contrast, we did not detect a significant difference in ATP levels comparing HIF1α^{flox} BMDCs under normoxic and hypoxic conditions, indicating that the reduced growth of WT BMDCs under hypoxia occurs independently of ATP production. A known target of HIF1α is the ectonucleotidase CD73, which cleaves extracellular AMP to adenosine [25]. We were able to show that CD73 is expressed at low levels on murine BMDCs and is upregulated under hypoxic conditions in an HIF1α-dependent manner (Fig. 2C). BMDCs of all three mouse strains analyzed showed increased

expression of major histocompatibility complex class II (MHC II) and CD86 when grown in hypoxia, while CD80 was only moderately enhanced (Fig. 2D and Supporting Information Fig. 2). In contrast to normoxic BMDCs, treatment of hypoxic BMDCs with lipopolysaccharide (LPS) could not further enhance the expression of maturation markers (data not shown). Because it is possible that reoxygenation of the cells occurred during the staining procedure [26], we also performed a control experiment in which cells were fed with hypoxic medium and fixed immediately after harvesting. Also, under these conditions, a similar upregulation of maturation markers was observed under hypoxia (Supporting Information Fig. 2). Thus, BMDCs differentiated under hypoxic conditions show a reduced growth rate but enhanced maturation compared with BMDCs raised under normoxia. The growth reduction, the ATP production, as well as the CD73 expression under hypoxia are furthermore dependent on HIF1α.

Altered production of cytokines in hypoxia

To assess the effector functions of BMDCs in hypoxia, we have analyzed cytokine production by BMDCs of cHIF1α^{CD11c}, cHIF1α^{CCL17}, and HIF1α^{flox} mice. We detected reduced amounts of interleukin (IL)-12p70, IL-10, IL-6, and IL-23 under hypoxic conditions in all mouse strains, irrespective of absence or presence of HIF1α. Additionally, production of tumor necrosis factor (TNF)-α and IL-1β was reduced in hypoxia in cHIF1α^{CD11c} and HIF1α^{flox} BMDCs. In hypoxic BMDCs of all three strains, we found IL-22 to be strongly upregulated compared with normoxic cells and this upregulation was at least in part HIF1α-dependent (Fig. 3A). By intracellular cytokine staining, we could show that the IL-12p40 protein was downregulated in an HIF1α-dependent manner under hypoxic conditions (Fig. 3B). Since IL-12 and IL–23 share the p40 subunit, we also analyzed the expression of IL-12p35, IL-23p19, and p40 by quantitative real-time PCR in cHIF1α^{CD11c} and HIF1α^{flox} mice. The expression of p40 mRNA was unaltered in cHIF1α^{CD11c} BMDCs compared with controls. The expression of p35, however, was reduced under hypoxic conditions in both cHIF1α^{CD11c} and HIF1α^{flox} BMDCs, which correlates with the reduced production of IL-12p70 protein. In contrast to IL-23 protein, the expression of IL-23p19 mRNA was strongly enhanced under hypoxic conditions (Fig. 3C). We conclude that hypoxia has a major influence on cytokine production, which is partly dependent on HIF1α.

Regulation of chemokines and chemokine receptors under hypoxic conditions

In case of inflammation and hence under hypoxic conditions, immune cells reorganize their chemokine production and chemokine receptor expression [14–17]. DCs migrate from the periphery to the draining LNs via a chemokine gradient after receipt of microbial or danger signals. The chemokine receptors CCR7 and CXCR4 are crucial for this process [13, 27]. Therefore, we analyzed the expression of chemokine receptors on BMDCs under hypoxia and normoxia. We found enhanced expression of CCR7 under hypoxic conditions compared with that found in normoxia. Notably, this upregulation was not detectable in HIF1α-deficient BMDCs. Expression of CXCR4 was generally low, but was slightly upregulated under hypoxia, depending on the presence of HIF1α. Expression of CCR5, a chemokine receptor involved in the recruitment of immature DCs from the blood to inflamed tissues, was barely detectable and was not significantly altered by hypoxia or by loss of HIF1α (Fig. 4A). Recently, we could show that the DC-derived chemokine CCL17 is also involved in the

migration of cutaneous DCs [20]. Therefore, the expression levels of CCL17 and CCL22, both ligands of CCR4, were measured in HIF1α-deficient and HIF1α^{flox} BMDC cultures. In all BMDC cultures, we found significantly higher levels of CCL17 and CCL22 in hypoxia compared with those in normoxic conditions (Fig. 4B). In cHIF1α^{CCL17} BMDCs, the production of CCL17 is generally reduced compared with that of control BMDCs, since cHIF1α^{CCL17} mice are heterozygous for the production of CCL17 due to integration of the Cre transgene [20]. Nevertheless, hypoxic upregulation of both chemokines was independent of HIF1α. These results show that conditions of diminished oxygen pressure clearly influence the expression of chemokines and chemokine receptors, indicating that hypoxia changes the migratory behavior of DCs.

Hypoxia leads to increased migration of BMDCs to CCL19 in an HIF1α-dependent manner

To assess the impact of hypoxia on the migratory capacity of BMDCs, we tested the ability of BMDCs to migrate toward CCL19 and CXCL12 in a transwell migration assay. As expected from the data on CCR7 surface expression, migration of BMDCs to CCL19 was enhanced in an HIF1α-dependent manner under hypoxic conditions. In contrast, migration toward the chemokine CXCL12 was neither affected by oxygen concentration nor by deficiency of HIF1α (Fig. 5A). To further examine the migratory capacity of BMDCs generated under hypoxic conditions, we performed an in vivo assay in which BMDCs were injected into the footpad and their immigration to the popliteal LNs was analyzed. To better compare the migration of BMDCs generated under normoxic or hypoxic conditions, as well as HIF1α-knockout versus WT BMDCs, we injected 1:1 mixtures of differentially labeled cells. Twentyfour hours later, the relative proportion of red- or green-labeled BMDCs in the popliteal LNs was determined (Fig. 5B and C). HIF1α-deficient and HIF1α^{flox} BMDCs grown in 21% oxygen migrated equally well to draining LNs (Fig. 5B, top left), whereas a significantly reduced migration of HIF1α-deficient BMDCs compared with HIF1α-proficient BMDCs was observed when the cells were generated under oxygen-deprived conditions (Fig 5B, top right). Furthermore, coinjection of HIF1α^{flox} BMDCs generated under normoxic or hypoxic conditions into footpads revealed a higher proportion of migrated HIF1α^{flox} BMDCs derived from hypoxic cultures (Fig. 5B, lower left). This enhanced migratory capacity was dependent on HIF1α, since HIF1α-deficient BMDCs generated under hypoxia migrated almost like normoxic HIF1α-deficient BMDCs (Fig. 5B, lower right). Thus, HIF1α appears to be required for the hypoxia-induced enhancement of the migratory capacity of BMDCs toward CCL19 in vitro and in vivo.

Discussion

Using conditional ablation of HIF1α in vivo, the functional importance of this transcription factor has already been examined in macrophages/granulocytes and T cells [6–9]. In this study, we analyzed the role of HIF1α for DC differentiation and function under hypoxic conditions. We found that hypoxia leads to enhanced phenotypic maturation of BMDCs but reduced cell growth and production of several cytokines, independent of HIF1α. In contrast, HIF1α-deficiency significantly diminished hypoxia-induced IL-22 production and expression of the chemokine receptor CCR7. Thus, our findings indicate that stabilization of

HIF1α in a low-oxygen environment specifically regulates cytokine expression and the ability of DCs to respond to certain chemotactic stimuli.

We employed two different Cre-transgenic lines, CD11cCre and CCL17Cre, for cell-type specific deletion of HIF1α in DCs. The cHIF1α^{CD11c} cross has the advantage of an efficient deletion of HIF1α in all DCs throughout the body, comprising migratory DCs from the periphery, tissue-resident DCs, and blood-circulating pDCs [28], as well as BMDCs generated in vitro. The disadvantage of this model is that the Cre-recombinase may also be active in a subset of T cells, as seen in the crosses to HIF1α^{flox} and RA/EG mice in this study, but to a lesser extent in other conditional mutant mice [18, 29, 30]. Therefore, phenotypic changes observed in in vivo studies of cHIF1α^{CD11c} mice may also result from T-cell-dependent effects [6–8]. Such T-cell-dependent alterations do not occur in cHIF1α^{CCL17} mice, as this line did not show deletion in the T-cell compartment (Fig. 1B). In the cHIF1α^{CCL17} line, however, HIF1α deletion affects only the CCL17-producing DC subset, which is mainly found in barrier organs such as skin, lung, and gut, as well as LNs [19]. In these tissues, in which hypoxic conditions may occur frequently, in particular in inflamed areas, CCL17^{+} DCs represent potent sentinels for incoming pathogens. In the present study, we mainly focused on the analysis of BMDCs, which are more efficiently deleted in the cHIF1α^{CD11c} background compared with the cHIF1α^{CCL17} background.

BMDCs generated under hypoxia showed impaired growth and a more mature phenotype defined by upregulation of MHC II and CD86. These results go in line with several recent studies in which it was shown that immature human monocyte derived DCs undergo maturation within a hypoxic environment [14, 16, 31], as well as another study that described an amplified LPS-induced maturation of mouse BMDCs under hypoxic conditions [32]. Thus, it appears that not only pathogen-associated molecular patterns (PAMPs) can act as danger signals to DCs, but also low oxygen concentrations as a consequence of inflammation. In other studies, however, hypoxia was found to either inhibit [26, 33] or not affect [34] the expression of maturation markers. This discrepancy may be explained by species differences, or differences in the specific experimental setup. The decreased proliferation/survival observed under hypoxia might result from the fact that the cells have to switch from aerobic oxidative phosphorylation to anaerobic glycolysis and undergo other changes in cell metabolism [2]. This may involve the activation of apoptotic pathways, although the role of HIF1α in hypoxia-induced apoptosis appears to vary between cell types [2, 3]. Thus, we cannot rule out that differentiation of DCs under hypoxic conditions may activate proapoptotic pathways. Alternatively, the hypoxic milieu might also favor differentiation of BMDCs, rather than proliferation. Though the absence of HIF1α did not play a role in the enhancement of maturation, proliferation/survival of HIF1α-deficient BMDCs was even lower than that of HIF1α^{flox} BMDCs under hypoxic conditions, probably resulting from a disturbed energy metabolism in the absence of HIF1α [2].

Regarding effector functions of DCs, we found that cytokine production was strongly influenced by low oxygen concentrations. We observed that the production of IL-12p70, IL-10, IL-6, TNF-α, IL-1β, and IL-23 was attenuated in hypoxia. Our findings differ from those of recent reports in which human monocyte derived DCs were shown to upregulate IL-10 [31] and the proinflammatory cytokines TNF-α and IL-1β under hypoxia [35]. In

another study, mouse BMDCs also produced higher levels of TNF and IL-6, when cultured under low oxygen concentrations [32]. In contrast, others have demonstrated similar decreases in cytokine production of human DCs as observed in our study [14]. It appears that the reduced cytokine production by hypoxic BMDCs is not simply resulting from a generally decreased protein translation as a consequence of disturbed energy metabolism, because certain chemokines and chemokine receptors were more strongly expressed under oxygen deprivation. Furthermore, IL-22 was strongly induced under hypoxia, in particular in the presence of HIF1α. IL-22 is known to induce antimicrobial peptides and proteins regulating cell mobility in nonimmune cells such as keratinocytes and epithelial cells [36]. Several studies have reported an important role for IL-22 in inflammation, especially in mucosal immunity, where IL-22 may also exert a protective role for the integrity of the epithelium [37]. IL-22 knockout mice were unable to efficiently clear intestinal infections with *Citrobacter rodentium* [38], and treatment of murine lungs with neutralizing antibodies against IL-22 during *Klebsiella pneumoniae* infection led to an increased bacterial burden [39]. IL-22 has been reported to be inducible by IL-23 in murine DCs [38]. Here, we show that the induction of this cytokine under hypoxic conditions is at least in part HIF1α-dependent. Therefore, induction of IL-22 in the course of infection may be enhanced by HIF1α as pathogen invasion often leads to a hypoxic environment, and also to NF-κB-dependent stabilization of HIF1α [5, 40].

Another prominent effect of HIF1α in BMDCs is the upregulation of CCR7 under hypoxic conditions. In agreement with this HIF1α-dependent change in chemokine receptor expression, we could demonstrate that $HIF1\alpha^{flox}$ BMDCs generated under hypoxia migrate to draining LNs more efficiently than normoxic BMDCs, and HIF1α-deficient BMDCs grown under hypoxic conditions exhibit impaired migration compared with $HIF1\alpha^{flox}$ BMDCs. As we do not know to which extend reoxygenation of the cells during the adoptive transfer influenced their migratory capacity, we cannot exclude that such reoxygenation alters the behavior of the cells. Nevertheless, our findings likely indicate that stabilization of HIF1α in inflammatory environments promotes emigration of DCs from the periphery into draining LNs. In lung cancer cells, CCR7 was also found to be upregulated in an HIF1α-dependent manner in hypoxia [41]. CXCR4 was previously identified as a target gene of HIF1α in many cell types [17], in agreement with our findings that CXCR4 expression in HIF1α-deficient BMDCs generated under conditions of low oxygen was slightly reduced compared with that of $HIF1\alpha^{flox}$ control cells. In contrast to our results, CCR5 was found to be upregulated and CCR7 downregulated on hypoxic human immature monocyte derived DCs [16, 35]. This discrepancy might be due to species-specific differences, or the use of different types of DCs. In addition to differential regulation of chemokine receptor expression, we found CCL17 and CCL22, two chemokines interacting with CCR4, to be secreted at higher levels in a hypoxic milieu. As shown previously by our group, CCL17 sensitizes DCs for efficient responsiveness to CCR7 ligands [20], so that migration of hypoxic DCs may be further enhanced by elevated production of CCL17.

Several of the phenotypic changes observed in hypoxic BMDCs, such as the enhanced maturation and the altered cytokine production, appeared to occur independent of HIF1α expression. This finding may be explained by the presence of another hypoxia inducible transcription factor, namely HIF2α. HIF1α and HIF2α are closely related, not only

structurally, but also concerning their binding to arylhydrocarbon receptor nuclear translocator (ARNT) and their ability to activate HRE-containing genes [42]. While HIF1α is ubiquitously expressed throughout the body, HIF2α has a more restricted expression pattern [43, 44]. In the case of the BMDCs used in this study, we were not able to detect expression of HIF2α mRNA, in contrast to BM-derived macrophages (data not shown). Nevertheless, we cannot exclude that HIF2α might in part compensate for the loss of HIF1α in BMDCs, although the two proteins may also have opposing effects [45]. In addition, several other signaling pathways besides HIF-dependent gene regulation, including NF-κB, were described to be involved in the response to hypoxia (reviewed in [46]).

In conclusion, we have shown that HIF1α enhances DC migration and production of IL-22 under hypoxic conditions, whereas elevated expression of MHC II and CD86, as well as diminished production of several other cytokines occur independently of HIF1α.

Material and methods

Mice

CCL17Cre mice were generated by insertion of CreNLS [47] and a neomycin cassette flanked by flippase recognition target (FRT)-recombination sites in the second exon of the *ccl17* gene. Homologously recombined embryonic stem cell clones (E14.1) were detected by Southern blot. The neomycin-resistance cassette was removed from the targeted *ccl17* locus by flippase (FLP) recombinase expression in vitro. After germline transmission, mice were backcrossed to the C57BL/6J background for more than eight generations.

DC-specific inactivation of HIF1α was achieved by crossbreeding CD11cCre [18] and CCL17Cre transgenic mice with $HIF1\alpha^{flox}$ mice [9]. CCL17/EGFP mice [19] and RA/EG reporter mice [24] were described previously. All mice were kept under pathogen-free conditions in the animal facility of the Leibniz Research Institute for Environmental Medicine. Animal experiments were done with permission of the government of North Rhine-Westphalia (AZ. 8.87–50.10.34.08.278).

Southern blot

To test deletion efficiency, a 227-bp HIF1α 3′ flanking probe was cloned using the primers CGGTGTGTATCATTCTATAG and CTGACATCAATACCTTCCCAC. The probe detects a 2.1 kb fragment of the HIF1α gene spanning exon 2, which is flanked by loxP sites. After Cre-mediated excision, a 1.5 kb fragment is detected. Genomic DNA was digested with *Eco*RI and *Pst*I. For calculation of the deletion efficiency, the intensity of the bands was quantified using the FluorChem 8900 software. The local background intensity was subtracted from the intensity of the specific bands, and the ratio of the normalized band intensities of the floxed and deleted band was calculated.

Cell sorting

To isolate CCL17-positive DCs for determination of Cre-mediated deletion efficiency, BMDCs were generated from $cHIF1\alpha^{CCL17}$ mice crossed to CCL17/EGFP reporter mice [19]. After stimulation of BMDCs with LPS, DCs expressing EGFP under the control of

ccl17 were sorted with a purity of more than 95% using an FACS Aria (BD Biosciences, Heidelberg, Germany).

Generation and in vitro stimulation of BMDCs

BM cells were plated at a concentration of 5×10^5 cells/mL in RPMI 1640 (Invitrogen), 10% FCS, L-glutamine, penicillin–streptomycin, and β-mercaptoethanol. For differentiation, 2% supernatant of granulocyte-macrophage colony-stimulating factor (GM-CSF) transfected X63Ag8–653-cells was added. BM cells were cultured under either normoxic (21% O_2) or hypoxic (1% O_2) conditions. On day 3, cells were fed with the same medium and harvested on day 6. For in vitro stimulation, BMDCs were plated at 1×10^6 cells/mL onto six-well plates (Greiner Bio-One, Solingen, Germany) and stimulated with 1 μg/mL LPS *Escherichia coli* 0111:B4 (Sigma-Aldrich, Munich, Germany) for 16–20 h.

Hypoxic conditions were obtained by culturing the cells in the presence of 1% O_2/5% CO_2/94% N_2 in an incubator with adjustable O_2 concentration (Hera Cell 150, Thermo Scientific, Schwerte, Germany). For the period of BMDC generation, LPS stimulation, as well as in vitro migration assays, cells were kept in the hypoxic incubator. For most experiments, cells were briefly treated under normoxic conditions for feeding on day 3 and kept on ice under normoxic conditions for all staining and centrifugation procedures. In some experiments, cells were fed with medium equilibrated under hypoxic conditions for 24 h before use and were immediately fixed in 1% paraformaldehyde prior to surface marker staining to prevent reoxygenation (Supporting Information Fig. 2).

Flow cytometry

Anti-MHC II (M5/114.15.2), anti-CD11c (N418), anti-CD80 (16–10A1), anti-CD86 (PO3.1), anti-CD3 (145–2C11), and anti-CCR7 (4B12) were purchased from eBioscience (Frankfurt, Germany); anti-CD11c (HL3), anti-CD73 (TY/23), anti-CCR5 (C34–3448), anti-CD8 (53–5.8), and Streptavidin-PE from BD Biosciences; anti-CXCR4 (247506) from R&D Systems (Wiesbaden-Nordenstadt, Germany), and anti-CD4 (RM4–5) from Bio Legend (Uithoorn, Netherlands). Cells were stained with the appropriate Ab in PBS on ice for 15 min and analyzed with an FACScalibur (BD Biosciences). Data were analyzed with FlowJo software (Tree star, Ashland, USA). For intracellular staining, cells were preincubated with GolgiStop (BD Biosciences), fixed, and permeabilized before staining with anti-IL-12p40/p70 (C15.6, BD Biosciences).

ATP measurement

BMDCs were harvested on day 6 and adjusted to 5×10^7 cells/mL. Intracellular ATP was quantified with the ATP Bioluminescence Assay Kit CLSII (Roche Diagnostics, Mannheim, Germany).

Enzyme-linked immunosorbent assay (ELISA)

Cell culture supernatants were analyzed for IL-1β, IL-10, IL-6, IL-22, IL-23, TNF-α, CCL17, and CCL22 using ELISA DuoSet Development kits (R&D Systems); IL-12p70 was detected with the Ready-SET-Go! Kit (eBioscience).

Quantitative real-time PCR

BMDCs were either stimulated or not with 1 μg/mL LPS for 3 h. RNA was isolated using the RNeasy mini kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized from 1 μg of total RNA using a mixture of oligo(dT) and Revert Aid reverse transcriptase (Fermentas, St.Leon-Rot, Germany). Absolute SYBR-green ROX (Thermo Scientific, Schwerte, Germany) mix was used to detect PCR products on a Rotor-Gene 3000 cycler (Qiagen). RNA expression levels were normalized to β-actin and displayed as fold change relative to normoxic, unstimulated $HIF1\alpha^{flox}$ cells used as calibrator (set to 1).

Migration assay

Migration of BMDCs was assessed using Transwell Chambers (8.0-μm pore diameter, Costar, Schwerte, Germany). The lower wells contained 700 μL of RPMI/0.5% FCS as control, or RPMI/0.5% FCS containing 200 ng/mL recombinant mouse CCL19/MIP-3β or 200 ng/mL recombinant mouse CXCL12/SDF-1α (both from R&D Systems). A total of 2×10^5 cells in 300 μL medium were added into the upper chambers and incubated at 37°C either under normoxic or under hypoxic conditions. After 4 h, cells in the lower chamber were counted and the proportion of $CD11c^+$ cells in the BMDC culture as well as the proportion of migrated $CD11c^+$ cells was assessed by fluorescence-activated cell sorter (FACS) analysis.

In vivo migration of BMDCs

BMDCs were labeled with CellTracker Probes for Long-Term Tracing of Living Cells (Invitrogen). Green CMFDA labeled BMDCs were mixed in equal amounts with orange CMTMR labeled BMDCs. In independent experiments, groups of BMDCs to be compared were labeled with one or the other fluorescent marker to exclude dye-specific influences. A total of 2×10^6 cells were injected into the footpad of C57BL/6 mice. Twenty-four hours later, popliteal LNs were dissected and analyzed by flow cytometry.

Statistical analysis

Statistical analysis of the data was performed using the Student's *t* test. All data are presented as mean + SEM. *p*-values < 0.05 were depicted as (*) or (#), $p < 0.01$ as (**) or (##), and $p < 0.001$ as (***) or (###) as indicated in the figure legends.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Ingo Uthe for technical assistance, to Philipp Dresing for help with cell sorting, and to Bernhard Homey for advice and discussion. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) through projects FO178/2–2 (FOR729) (I.F.), SFB 704 (A1) (I.F.), and GK1427 (Project 9) (I.F. and Roel Schins).

Abbreviations

BMDC	bone marrow derived dendritic cell

EGFP	enhanced green fluorescent protein
GM-CSF	granulocyte-macrophage colony-stimulating factor
HIF1α	hypoxia-inducible factor 1 alpha
HRE	hypoxia-responsive element
MHC II	MHC class II
MFI	mean fluorescence intensity

References

1. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol. 1992; 12:5447–5454. [PubMed: 1448077]
2. Sitkovsky M, Lukashev D. Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. Nat Rev Immunol. 2005; 5:712–721. [PubMed: 16110315]
3. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. Nat Rev Immunol. 2009; 9:609–617. [PubMed: 19704417]
4. Zinkernagel AS, Johnson RS, Nizet V. Hypoxia inducible factor (HIF) function in innate immunity and infection. J Mol Med. 2007; 85:1339–1346. [PubMed: 18030436]
5. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, et al. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 1998; 12:149–162. [PubMed: 9436976]
6. Thiel M, Caldwell CC, Kreth S, Kuboki S, Chen P, Smith P, Ohta A, et al. Targeted deletion of HIF-1alpha gene in T cells prevents their inhibition in hypoxic inflamed tissues and improves septic mice survival. PLoS One. 2007; 2:e853. [PubMed: 17786224]
7. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell. 2011; 146:772–784. [PubMed: 21871655]
8. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, Chi H. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med. 2011; 208:1367–1376. [PubMed: 21708926]
9. Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, et al. HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell. 2003; 112:645–657. [PubMed: 12628185]
10. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000; 18:767–811. [PubMed: 10837075]
11. Steinman RM. Dendritic cells: understanding immunogenicity. Eur J Immunol. 2007; 37(Suppl 1):S53–60. [PubMed: 17972346]
12. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. Nat Rev Immunol. 2007; 7:19–30. [PubMed: 17170756]
13. Randolph GJ, Ochando J, Partida-Sanchez S. Migration of dendritic cell subsets and their precursors. Annu Rev Immunol. 2008; 26:293–316. [PubMed: 18045026]
14. Elia AR, Cappello P, Puppo M, Fraone T, Vanni C, Eva A, Musso T, et al. Human dendritic cells differentiated in hypoxia down-modulate antigen uptake and change their chemokine expression profile. J Leukoc Biol. 2008; 84:1472–1482. [PubMed: 18725395]
15. Bosco MC, Puppo M, Blengio F, Fraone T, Cappello P, Giovarelli M, Varesio L. Monocytes and dendritic cells in a hypoxic environment: spotlights on chemotaxis and migration. Immunobiology. 2008; 213:733–749. [PubMed: 18926289]
16. Ricciardi A, Elia AR, Cappello P, Puppo M, Vanni C, Fardin P, Eva A, et al. Transcriptome of hypoxic immature dendritic cells: modulation of chemokine/receptor expression. Mol Cancer Res. 2008; 6:175–185. [PubMed: 18314479]

17. Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, et al. Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med. 2003; 198:1391–1402. [PubMed: 14597738]
18. Caton ML, Smith-Raska MR, Reizis B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. J Exp Med. 2007; 204:1653–1664. [PubMed: 17591855]
19. Alferink J, Lieberam I, Reindl W, Behrens A, Weiss S, Huser N, Gerauer K, et al. Compartmentalized production of CCL17 in vivo: strong inducibility in peripheral dendritic cells contrasts selective absence from the spleen. J Exp Med. 2003; 197:585–599. [PubMed: 12615900]
20. Stutte S, Quast T, Gerbitzki N, Savinko T, Novak N, Reifenberger J, Homey B, et al. Requirement of CCL17 for CCR7- and CXCR4-dependent migration of cutaneous dendritic cells. Proc Natl Acad Sci USA. 2010; 107:8736–8741. [PubMed: 20421491]
21. Bedogni B, Welford SM, Cassarino DS, Nickoloff BJ, Giaccia AJ, Powell MB. The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. Cancer Cell. 2005; 8:443–454. [PubMed: 16338658]
22. Taylor CT, Colgan SP. Hypoxia and gastrointestinal disease. J Mol Med. 2007; 85:1295–1300. [PubMed: 18026919]
23. Vinay DS, Kim CH, Choi BK, Kwon BS. Origins and functional basis of regulatory CD11c+CD8+ T cells. Eur J Immunol. 2009; 39:1552–1563. [PubMed: 19499519]
24. Constien R, Forde A, Liliensiek B, Grone HJ, Nawroth P, Hammerling G, Arnold B. Characterization of a novel EGFP reporter mouse to monitor Cre recombination as demonstrated by a Tie2 Cre mouse line. Genesis. 2001; 30:36–44. [PubMed: 11353516]
25. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, Hansen KR, et al. Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. J Clin Invest. 2002; 110:993–1002. [PubMed: 12370277]
26. Wang Q, Liu C, Zhu F, Liu F, Zhang P, Guo C, Wang X, et al. Reoxygenation of hypoxia-differentiated dentritic cells induces Th1 and Th17 cell differentiation. Mol Immunol. 2010; 47:922–931. [PubMed: 19910049]
27. Kabashima K, Shiraishi N, Sugita K, Mori T, Onoue A, Kobayashi M, Sakabe J, et al. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. Am J Pathol. 2007; 171:1249–1257. [PubMed: 17823289]
28. Naik SH. Demystifying the development of dendritic cell subtypes, a little. Immunol Cell Biol. 2008; 86:439–452. [PubMed: 18414430]
29. Sathaliyawala T, O'Gorman WE, Greter M, Bogunovic M, Konjufca V, Hou ZE, Nolan GP, et al. Mammalian target of rapamycin controls dendritic cell development downstream of Flt3 ligand signaling. Immunity. 2010; 33:597–606. [PubMed: 20933441]
30. Hou B, Reizis B, DeFranco AL. Toll-like receptors activate innate and adaptive immunity by using dendritic cell-intrinsic and -extrinsic mechanisms. Immunity. 2008; 29:272–282. [PubMed: 18656388]
31. Rama I, Bruene B, Torras J, Koehl R, Cruzado JM, Bestard O, Franquesa M, et al. Hypoxia stimulus: an adaptive immune response during dendritic cell maturation. Kidney Int. 2008; 73:816–825. [PubMed: 18216782]
32. Jantsch J, Chakravortty D, Turza N, Prechtel AT, Buchholz B, Gerlach RG, Volke M, et al. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. J Immunol. 2008; 180:4697–4705. [PubMed: 18354193]
33. Yang M, Ma C, Liu S, Sun J, Shao Q, Gao W, Zhang Y, et al. Hypoxia skews dendritic cells to a T helper type 2-stimulating phenotype and promotes tumour cell migration by dendritic cell-derived osteopontin. Immunology. 2009; 128:e237–e249. [PubMed: 19740309]
34. Bosco MC, Pierobon D, Blengio F, Raggi F, Vanni C, Gattorno M, Eva A, et al. Hypoxia modulates the gene expression profile of immunoregulatory receptors in human mature dendritic cells: identification of TREM-1 as a novel hypoxic marker in vitro and in vivo. Blood. 2011; 117:2625–2639. [PubMed: 21148811]
35. Mancino A, Schioppa T, Larghi P, Pasqualini F, Nebuloni M, Chen IH, Sozzani S, et al. Divergent effects of hypoxia on dendritic cell functions. Blood. 2008; 112:3723–3734. [PubMed: 18694997]

36. Wolk K, Witte E, Witte K, Warszawska K, Sabat R. Biology of interleukin-22. Semin Immunopathol. 2010; 32:17–31. [PubMed: 20127093]
37. Zenewicz LA, Flavell RA. IL-22 and inflammation: leukin' through a glass onion. Eur J Immunol. 2008; 38:3265–3268. [PubMed: 19016525]
38. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med. 2008; 14:282–289. [PubMed: 18264109]
39. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med. 2008; 14:275–281. [PubMed: 18264110]
40. Taylor CT, Cummins EP. The role of NF-kappaB in hypoxia-induced gene expression. Ann NY Acad Sci. 2009; 1177:178–184. [PubMed: 19845620]
41. Li Y, Qiu X, Zhang S, Zhang Q, Wang E. Hypoxia induced CCR7 expression via HIF-1alpha and HIF-2alpha correlates with migration and invasion in lung cancer cells. Cancer Biol Ther. 2009; 8:322–330. [PubMed: 19305150]
42. Loboda A, Jozkowicz A, Dulak J. HIF-1 and HIF-2 transcription factors–similar but not identical. Mol Cells. 2010; 29:435–442. [PubMed: 20396958]
43. Imtiyaz HZ, Williams EP, Hickey MM, Patel SA, Durham AC, Yuan LJ, Hammond R, et al. Hypoxia-inducible factor 2alpha regulates macrophage function in mouse models of acute and tumor inflammation. J Clin Invest. 2010; 120:2699–2714. [PubMed: 20644254]
44. Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Mol Cell Biol. 2003; 23:9361–9374. [PubMed: 14645546]
45. Takeda N, O'Dea EL, Doedens A, Kim JW, Weidemann A, Stockmann C, Asagiri M, et al. Differential activation and antagonistic function of HIF-{alpha} isoforms in macrophages are essential for NO homeostasis. Genes Dev. 2010; 24:491–501. [PubMed: 20194441]
46. Nakayama K. Cellular signal transduction of the hypoxia response. J Biochem. 2009; 146:757–765. [PubMed: 19864435]
47. Gu H, Zou YR, Rajewsky K. Independent control of immunoglobulin switch recombination at individual switch regions evidenced through Cre-loxP-mediated gene targeting. Cell. 1993; 73:1155–1164. [PubMed: 8513499]

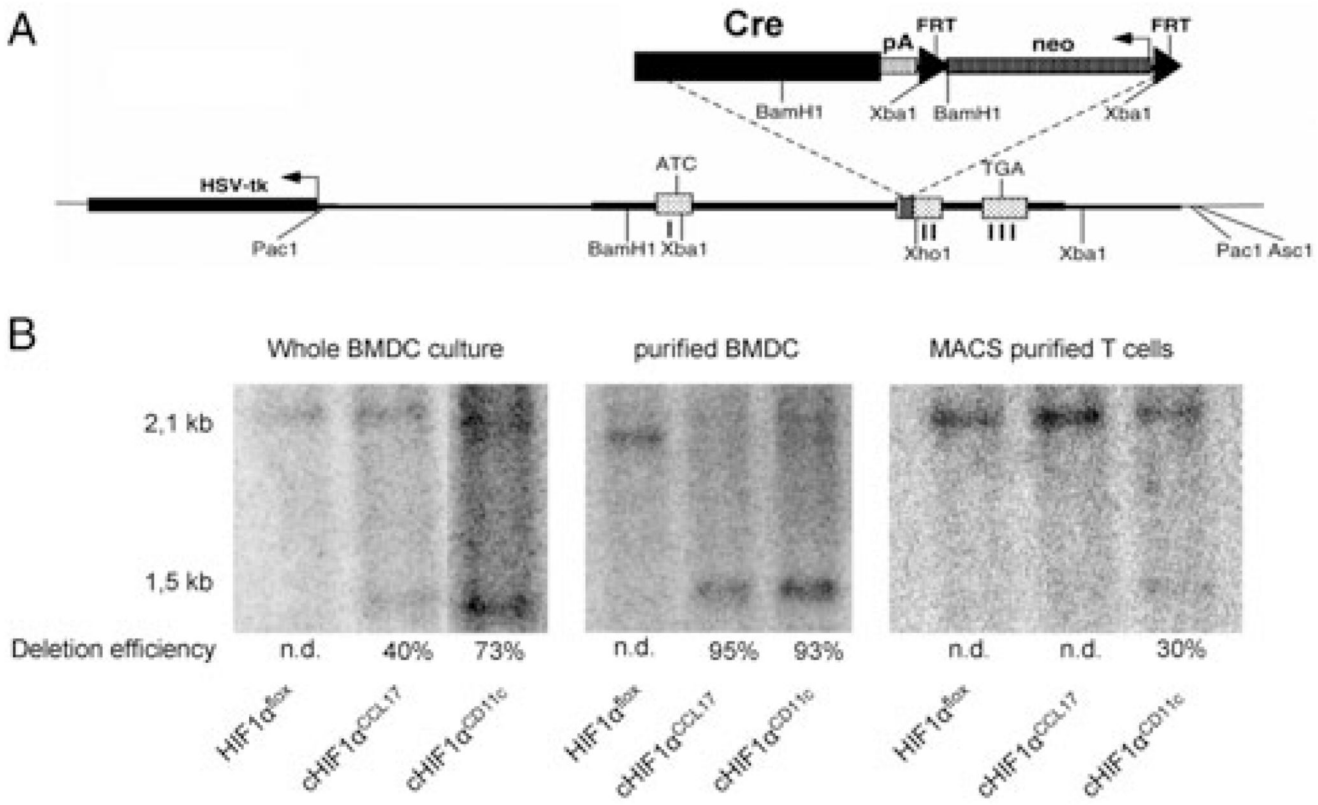

Figure 1.

Conditional knockout of hypoxia-inducible transcription factor (HIF)1α. (A) Targeting strategy for integration of Cre in the second exon of the ccl17 gene. (B) Efficiency of HIF1α deletion was tested by Southern blot. A ^{32}P-labeled HIF1α probe was hybridized to DNA from whole bone marrow derived dendritic cell (BMDC) cultures (left), as well as to fluorescence-activated cell sorter (FACS) sorted CCL17-positive cells from BMDCs of CCL17/EGFP reporter [19] × cHIF1α^{CCL17} mice and MACS-purified CD11c-positive cells from cHIF1α^{CD11c} BMDCs (middle). MACS-sorted CD3-positive T cells were also analyzed for HIF1α deletion (right). Fragments with the size of 2.1 kb represent intact HIF1α, whereas the 1.5-kb band indicates Cre-mediated deletion. Data are representative of two to three independent experiments, except for analysis of FACS-sorted cells (one experiment).

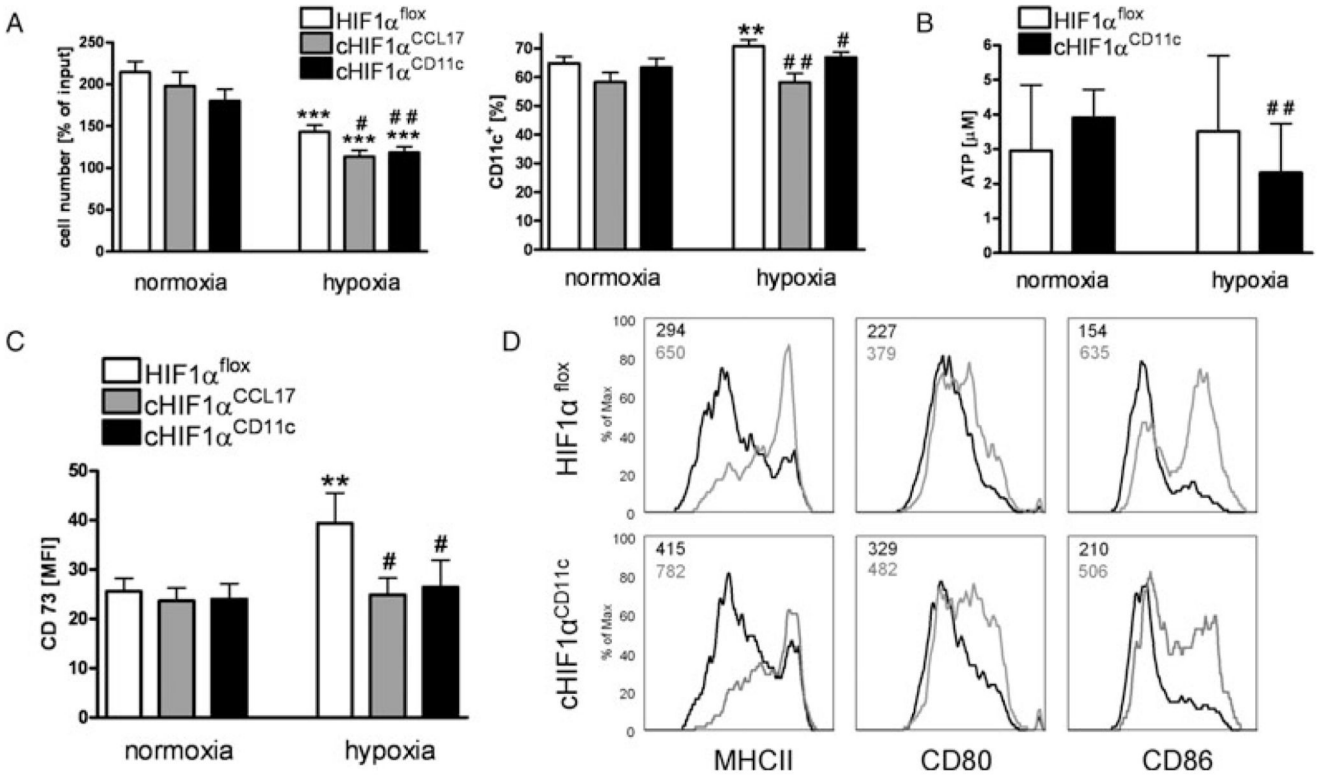

Figure 2.

Reduced growth and enhanced maturation of BMDCs under hypoxia. BMDCs of HIF1α^{flox}, cHIF1α^{CCL17}, and cHIF1α^{CD11c} mice were cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF) at 21% or 1% O_2. (A) On day 6, cells were counted and the percent increase over input cells was calculated. The percentage of DCs in BM cultures was assessed by CD11c staining. Results are depicted as mean + SEM of n = 26–51 samples pooled from at least 26 experiments (cell numbers, left) and n = 11–16 samples pooled from at least 11 independent experiments (frequency of CD11c$^+$ cells, right). (B) Intracellular ATP was measured after cell lysis using a luciferase-based assay. Results are depicted as mean + SEM of n = 9 samples pooled from nine independent experiments. (C) Mean fluorescence intensity (MFI) of CD73 staining on gated CD11c-positive BMDCs grown under normoxic or hypoxic conditions. Results are depicted as mean + SEM of n = 10–15 samples pooled from at least ten independent experiments. (D) Hypoxic BMDCs (gray line histograms) and normoxic BMDCs (black line histograms) were analyzed by flow cytometry. Histograms depict expression of major histocompatibility complex (MHC) II (left), CD80 (middle), and CD86 (right) on gated CD11c-positive BMDCs. Values in the histograms indicate the MFI. Results are representative of at least three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, hypoxic versus normoxic cells; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, HIF1α-deficient BMDCs versus HIF1α^{flox} controls; Student's t test.

Europe PMC Funders Author Manuscripts

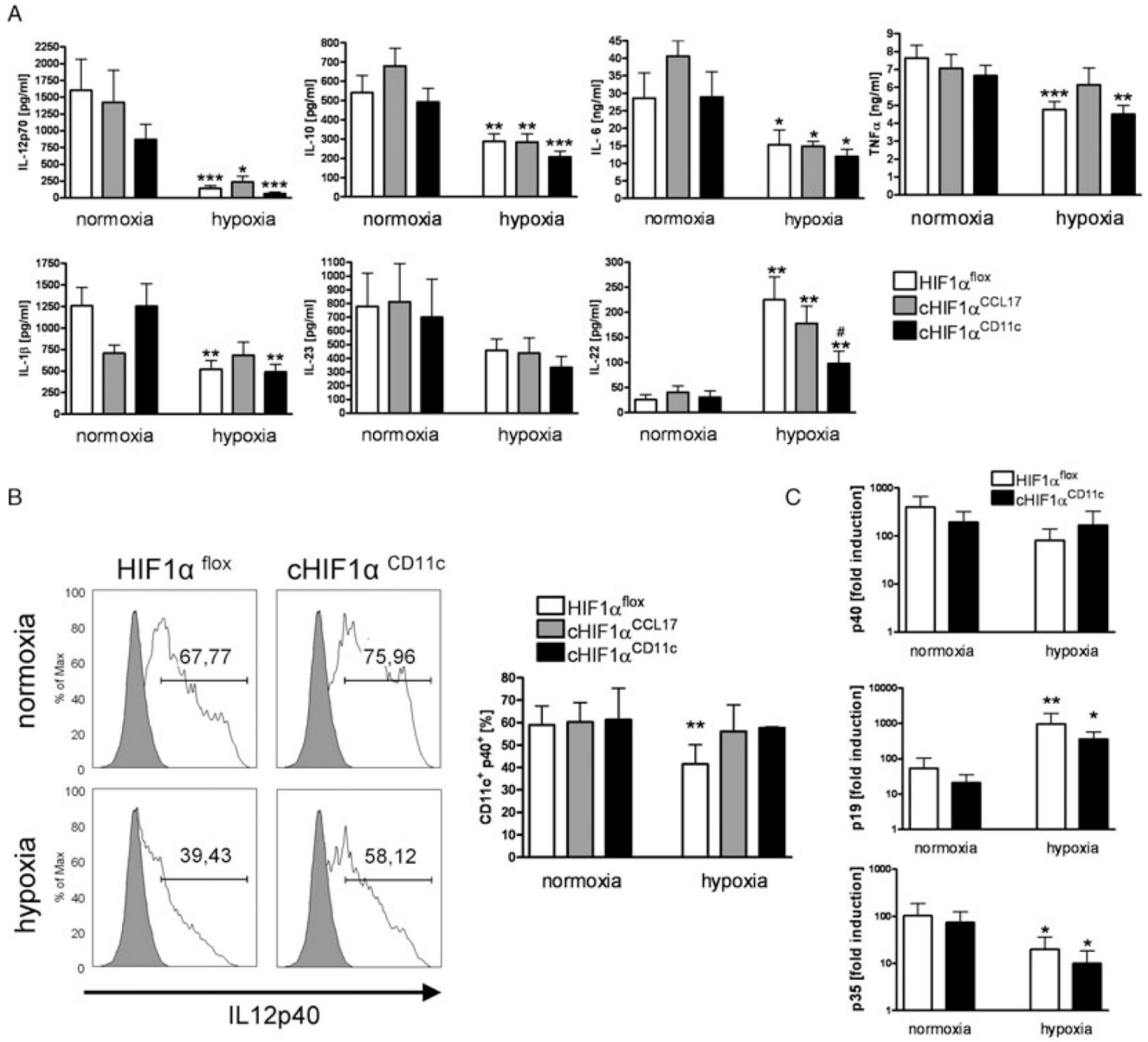

Figure 3.

Altered production of cytokines in hypoxia. BMDCs of HIF1α^{flox}, cHIF1α^{CCL17}, and cHIF1α^{CD11c} mice were cultured with GM-CSF at 21% (normoxia) or 1% O_2 (hypoxia). (A, B) On day 6, BMDCs were stimulated with lipopolysaccharide (LPS) for 16–20 h. (A) Cytokine concentrations in culture supernatants were measured by enzyme-linked immunosorbent assay (ELISA). Results are depicted as mean + SEM of n = 9–24 samples, except for interleukin (IL)-6 (n = 4–5 samples) pooled from at least four independent experiments. (B) Intracellular cytokine staining for IL12p40 on gated CD11c-positive BMDCs. The proportion of IL12p40-positive cells was determined in comparison with the negative control (gray shaded histogram) as indicated. Histograms are representative of at least six independent experiments. Bar graph (right) shows mean + SEM of all experiments

performed. (C) RNA was isolated 3 h after LPS stimulation and expression of p40 (top), p19 (middle), and p35 (bottom) was determined by relative quantification. Expression levels of unstimulated normoxic wild-type cells were set to 1. Results are depicted as mean + SEM of $n = 4–6$ samples pooled from at least three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ hypoxic versus normoxic cells; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.00$; HIF1α-deficient BMDCs versus HIF1α^{flox} controls; Student's *t* test.

Europe PMC Funders Author Manuscripts

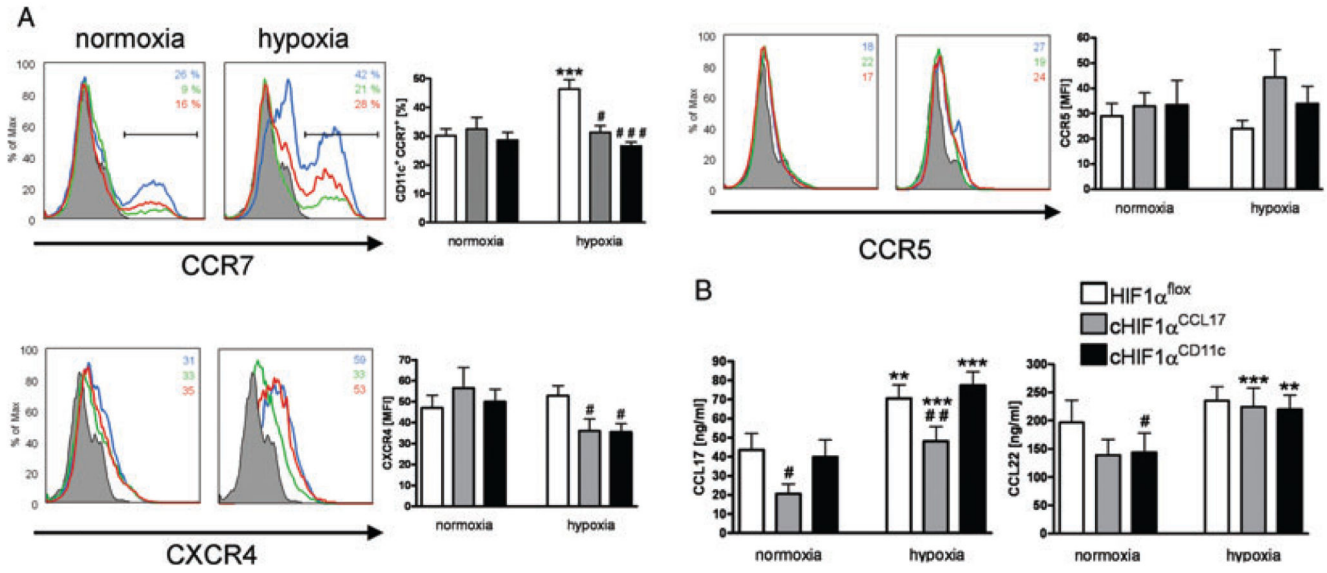

Figure 4.

Regulation of chemokines and chemokine receptors under hypoxic conditions. BMDCs of HIF1α^{flox}, cHIF1α^{CCL17}, and cHIF1α^{CD11c} mice were cultured with GM-CSF at 21% (normoxia) or 1% O_2 (hypoxia). (A) Gated CD11c-positive cells were stained with anti-CCR7, anti-CXCR4, or anti-CCR5. cHIF1α^{CD11c} BMDCs (red lines) and cHIF1α^{CCL17} BMDCs (green lines) were compared with HIF1α^{flox} BMDCs (blue lines). The numbers in the representative histograms indicate percentage of CCR7-positive cells (top), or the MFI of the CXCR4- and CCR5-staining (middle and bottom). The isotype control staining is shown as a gray shaded histogram. Bar graphs (right) depict mean + SEM of $n = 4–12$ samples pooled from at least three independent experiments. (B) Supernatants of LPS-stimulated cultures were tested for the presence of CCL17 (left) and CCL22 (right) by ELISA. Results are depicted as mean + SEM of $n = 9–14$ samples pooled from at least nine independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ hypoxic versus normoxic cells; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, HIF1α-deficient BMDCs versus HIF1α^{flox} controls; Student's t test.

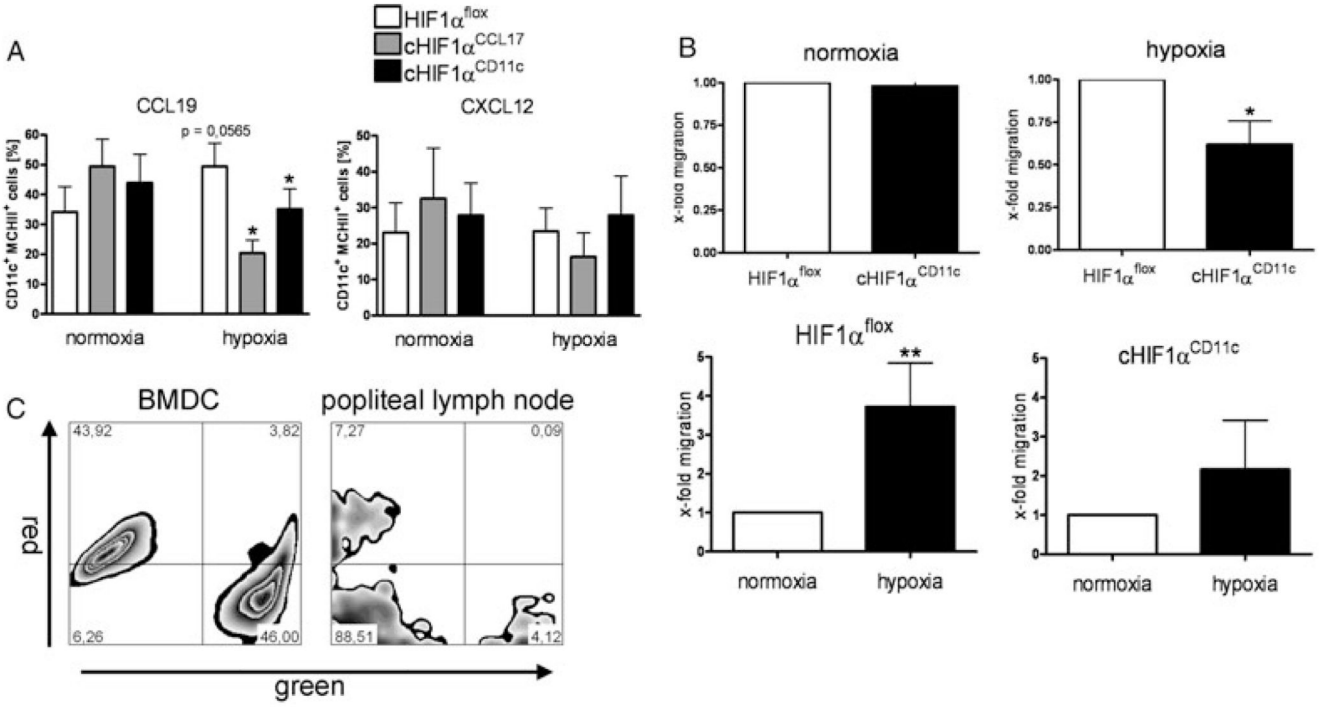

Figure 5.

Enhanced migration of hypoxic BMDCs is HIF1α-dependent. BMDCs of HIF1α^{flox}, cHIF1α^{CCL17}, and cHIF1α^{CD11c} mice were cultured with GM-CSF at 21% (normoxia) or 1% O_2 (hypoxia). (A) Migration of BMDCs toward the chemokines CCL19 (left) and CXCL12 (right) was examined in transwell chamber assays. Migrated cells were counted and stained with anti-CD11c and anti-MHC II to calculate the percentage of migrated DCs. Results are depicted as mean + SEM of $n = 3–6$ samples pooled from at least three independent experiments. (B) Green CMFDA-labeled and red CMTMR-labeled BMDCs were mixed in equal proportions and injected in the footpad. After 24 h proportions of differentially labeled CD11c-positive BMDCs in popliteal lymph nodes (LNs) were analyzed by flow cytometry as indicated in (C) and the ratio of migrated HIF1α^{flox} BMDCs (set to 1) compared with cHIF1α^{CD11c} BMDCs was determined for cells grown either under normoxic (left, top) or under hypoxic conditions (left, bottom). In addition, the ratio of migrated normoxic BMDCs (set to 1) compared with hypoxic BMDCs was determined for HIF1α^{flox} BMDCs (right, top), or for cHIF1α^{CD11c} BMDCs (right, bottom). Results are depicted as mean + SEM of $n = 5–7$ samples pooled from at least five independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; HIF1α-deficient BMDCs versus HIF1α^{flox} controls (left) or hypoxic versus normoxic cells (right); Student's t test.

Europe PMC Funders Author Manuscripts