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Hypoxia-inducible factor-1 α regulation of myeloid cells

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Abstract

Hematopoietic myeloblasts give rise to macrophages, dendritic cells, and neutrophils. Circulating myeloid cells detect invading microbes using pattern recognition receptors and subsequently orchestrate an innate immune response to contain and kill the pathogens. This innate immune response establishes an inflammatory niche characterized by hypoxia due to host and pathogen factors. Hypoxia-inducible factor (HIF) transcription factors are the primary regulators of the myeloid response to hypoxia. In particular, HIF-1 α is a critical hub that integrates hypoxic and immunogenic signals during infection or inflammation. Hypoxia induces HIF-1 α stabilization, which drives metabolic and phenotypic reprogramming of myeloid cells to maximize antimicrobial potential. HIF-1 α activity in myeloid-derived cells enhances the host response to infection but may also play a role in pathogenic inflammatory processes such as atherosclerosis. In this review, we summarize recent advances that have elucidated the mechanism by which myeloid cells regulate HIF-1 α activity and, in turn, how HIF-1 α shapes myeloid cell function.

Keywords

Myeloid; HIF; immunometabolism; hypoxia; inflammation

Introduction

Upon detection of a foreign pathogen, the innate immune system initiates a rapid and broad defense that contains the spread of the pathogen and subsequent cellular damage [1]. Most innate immune cells are derived from the myeloid hematopoietic lineage, which includes granulocytes, monocytes, macrophages, and dendritic cells [1–3]. Myeloid cells detect foreign products or host damage via activation of pattern recognition receptors, such as toll-like receptors (TLRs), induced by the binding of common pathogen-associated molecular patterns. This interaction triggers intracellular signaling cascades that initiate a proinflammatory response, consisting of increased production and release of inflammatory mediators, leukocyte recruitment, and microbial killing [4]. The character of the myeloid cell response depends on the microenvironment where detection of the pathogen or damage

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Conflicts of Interest

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occurs [5]. Areas of infection or sterile inflammation have a lower oxygen tension (hypoxia) than uninflamed tissues due to an increase in oxygen consumption by both host and microbial cells. In turn, myeloid cells have adopted mechanisms that promote antimicrobial function during hypoxia [6].

Hypoxia alters the transcription of hundreds of genes that allow cells to adapt to the environment [7]. Additionally, reduction of adenosine triphosphate (ATP) concentration and increase in reactive oxygen species (ROS) further modulate the transcriptional profile of hypoxic cells [8]. This is mediated in part by pleiotropic transcription factors such as activating protein-1 and cyclic adenosine monophosphate responsive element binding protein that converge on essential hypoxia response genes such as vascular endothelial growth factor (VEGF) [7]. However, the family of hypoxia-inducible factor (HIF) transcription factors are the primary regulators of the hypoxic response in all cells [9]. In myeloid cells, both HIF-1 and HIF-2 regulate cellular metabolism and antimicrobial function [6,9–11]. HIF-1 is a heterodimer of HIF-1 α and HIF-1 β subunits [12–14]. While HIF-1 β is constitutively expressed, the availability of HIF-1 α is tightly regulated by hypoxia.

Recent studies have uncovered the profound influence of HIF-1 activity on the differentiation, polarization, and activation of myeloid cells. Cellular hypoxia leads to stabilization of HIF-1 α , which dimerizes with HIF-1 β and translocates to the nucleus to modulate transcription of genes with promoters containing HIF response elements (HREs) (Figure 1) [15]. Important genes with HRE-containing promoters include glycolytic enzymes, VEGF, and TLRs [10,16]. Additionally, HIF-1 α activity enhances antimicrobial functions such as phagocytosis by increasing transcription of metabolic enzymes that can fuel such processes [17]. While HIF-1 has been established as a critical component of the myeloid hypoxic response, less is known about HIF-2. Loss of HIF-2 causes defects in myeloid migration and cytokine secretion in the setting of inflammation or malignancy [11,18]. Additionally, HIF-2 specifically regulates neutrophil survival, but does not impact antimicrobial capacity [19]. Interestingly, HIF-2 regulates myeloid function independently of cellular ATP concentration [11]. Further research is needed to identify the molecular targets of HIF-2, distinguish differential effects of HIF-1 and HIF-2, and contextualize the role of HIF-2 during infection.

This review details key advances in knowledge of the role of HIF-1 α in pathologic inflammatory states, with a particular focus on myeloid metabolism and antimicrobial function during infection. While the role of HIF-1 α in inflammation has been reviewed elsewhere recently, we provide a strong assessment of the unique mechanisms regulating HIF-1 α activity in myeloid cells. We also compare and contrast HIF-1 α activity and regulation across various myeloid-derived cells. Finally, we conclude with a discussion of the remaining gaps in knowledge of HIF-1 α and summarize avenues of potential therapeutic intervention.

Mechanisms of HIF-1 α Stabilization, Activation, and Degradation in Myeloid Cells

An array of factors contributes to the development of hypoxia at sites of inflammation. Infecting microbes disrupt microvascular circulation leading to decreased O₂ delivery to tissues [20]. Resident and immune cells generate ROS to kill pathogens, which is a process that consumes oxygen [21]. Additionally, the rapid influx of metabolically active innate immune cells triggers a rise in oxygen consumption that renders the microenvironment increasingly hypoxic [8]. As myeloid cells are recruited to sites of inflammation, they undergo a transition from the oxygen-rich vasculature to the hypoxic niche. This abrupt decrease in O₂ content inhibits prolyl-4-hydroxylase domain enzymes (PHDs), which hydroxylate proline residues on HIF-1 α in an oxygen-dependent manner. Hydroxylated HIF-1 α is targeted for ubiquitination by the von Hippel-Lindau E3 ubiquitin ligase (pVHL) and subsequently degraded by the proteasome (Figure 1) [22]. Thus, normal or elevated O₂ levels suppress, while hypoxia promotes, HIF-1 activation.

Exposure to the TLR4 agonist lipopolysaccharide (LPS; endotoxin) triggers HIF-1 α stabilization in myeloid cells [23–25]. While this has been known for over a decade, the mechanisms that mediate LPS-driven HIF-1 α stabilization have only recently been elucidated. Tannahill *et al.* showed that TLR4 activation increases concentrations of the metabolic intermediate succinate by way of sustained glutamine flux into the TCA cycle [26]. Succinate directly stabilizes HIF-1 α and promotes secretion of interleukin (IL)-1 β , a potent proinflammatory cytokine. Additionally, Rius *et al.* showed that the nuclear factor of κ B (NF- κ B) family of transcription factors is required for adequate HIF-1 α during inflammation. They found that the inhibitor of nuclear factor kappa B (I κ B) kinase β (IKK- β), activated by TLR4 signaling, plays a critical role in HIF-1 α stabilization under basal conditions and during inflammation by phosphorylating I κ B, thus mediating inflammation by licensing activation of NF- κ B [27]. *IKK- β ^{-/-}* macrophages fail to increase expression of HIF-1 α target genes after treatment with the hypoxia-mimetic deferoxamine, as compared to wildtype counterparts. Interestingly, hypoxia increases NF- κ B activity in the absence of TLR4 stimulation. Cummins *et al.* demonstrated that PHDs suppress IKK- β activity during normoxia by O₂-dependent hydroxylation of proline191 in an essential activation loop of the protein [28]. Hypoxia or genetic alteration of proline-191 is sufficient to induce NF- κ B nuclear translocation and activity. Importantly, HIF-1 α is not required for this phenomenon, suggesting that hypoxia directly liberates IKK- β from hydroxylation by PHD enzymes. Because NF- κ B promotes transcription of *HIF1A* mRNA, this pathway ensures adequate HIF-1 α protein is available to bind with HIF-1 β and respond appropriately to the hypoxia. Thus, myeloid cells employ synergistic pathways that increase both HIF-1 α synthesis and stabilization upon arrival at hypoxic inflammatory niches.

Myeloid cells also possess mechanisms that limit HIF-1 α activation to prevent pathologic inflammation. For example, the Protein Factor Inhibiting HIF-1 (FIH-1) hydroxylates asparagine residues on the C-terminal activation domain of HIF-1 α and prevents its interaction with the p300 coactivator protein [29,30]. Sakamoto and Seiki identified Mint3 as a novel binding partner of FIH-1 in myeloid cells [31]. They showed that knockdown of

Mint3, a constitutively expressed protein in the Golgi membrane, releases FIH-1 to translocate into the cytoplasm where it diminishes HIF-1 α activity and glycolysis, thus decreasing overall ATP production. Additionally, work from Hara *et al.* demonstrated that *Mint3*^{-/-} mice are resistant to LPS-induced septic shock due to a reduction in macrophage glycolysis, cytokine production, and motility [32]. FIH-1 may also modulate HIF-1 α activity and inflammation by hydroxylation of I κ B. Cockman *et al.* discovered that I κ B contains an ankyrin repeat domain that is susceptible to FIH-1 hydroxylation [33]. Manipulation of FIH-1 activity does not affect NF- κ B nuclear localization or activation after cytokine stimulation in human embryonic kidney cells. However, Shin and colleagues reported that the I κ B-FIH interaction enhances HIF-1 α activity in conditions of moderate hypoxia (1% O₂) [34]. Additionally, sirtuin deacetylase 1 and 2 (SIRT1/2) are known to limit inflammation by transitioning myeloid metabolism from glycolysis toward fatty acid oxidation (FAO), which prevents NF- κ B and inflammasome activation [35–37]. More recently, Seo *et al.* found that SIRT2 also destabilizes HIF-1 α by deacetylating lysine-709, which increases the binding affinity of PHD2 for HIF-1 α [38].

A multitude of signals converge on HIF-1 α to dictate its activity on a continuous scale. This raises the tantalizing possibility that HIF-1 α may serve as a rheostat to control levels of inflammation, opening the door for therapeutic targeting. Hams *et al.* utilized the hydroxylase inhibitor dimethyloxallyl glycine (DMOG), which increases HIF-1 α activity *in vitro*, to test this hypothesis in murine models of sepsis [39,40]. DMOG was found to improve survival in endotoxic shock, in part by skewing activation of macrophages to a less inflammatory phenotype [40]. Surprisingly, DMOG decreased LPS-induced NF- κ B activity in peritoneal myeloid cells despite theoretically increasing HIF-1 α activity. They also showed that DMOG decreases survival in a cecal ligation and puncture model of polymicrobial sepsis. While the authors did not directly measure HIF-1 α activity, this demonstrates that simply increasing or decreasing HIF-1 α activity broadly *in vivo* might not be a viable therapeutic strategy to combat infection. Understanding the specific roles HIF-1 α plays in different cell types may reveal unique targets for intervention. In the following sections, we discuss what is currently known about those specific roles in myeloid cells, including monocytes and macrophages, neutrophils, and dendritic cells.

HIF-1 α Regulation in Macrophages and Monocytes

Macrophages are critical effectors of innate immunity that mediate both pro- and anti-inflammatory programs. Macrophages can either be tissue-resident, maintained through self-renewal throughout life, or differentiate from monocytes that have extravasated from the bloodstream [41,42]. Depending on local stimuli, macrophages display a widely heterogeneous phenotype [43]. Macrophages exposed to bacterial products (e.g. LPS) or cytokines (e.g. IFN- γ) adopt a proinflammatory and microbiocidal phenotype that is heavily dependent on HIF-1 α activity [25,44,26,27,45]. Conversely, cytokines such as IL-4 and IL-13 lead macrophages toward a tissue repair phenotype with suppressed HIF-1 α activity [46].

Nearly 50 years ago, Hard discovered that proinflammatory macrophages consume glucose more rapidly than unstimulated cells [47]. However, the functional importance of increased

glycolysis was unknown until recently. Freerman *et al.* showed that overexpression of glucose transporter 1 (GLUT1), the primary transporter used by macrophages, is sufficient to induce proinflammatory cytokine secretion, enrichment of inflammatory gene expression, and production of ROS [48]. Haschemi and colleagues directly linked these findings to inflammation by showing that LPS-stimulated macrophages increase lactate production and flux through the pentose phosphate pathway (PPP), a biosynthetic metabolic pathway downstream of glycolysis [49]. Decades later, we now understand that HIF-1 α mediates these metabolic changes.

Numerous studies have implicated metabolic enzymes and intermediates in the stabilization of HIF-1 α . The final step of glycolysis is catalyzed by pyruvate kinase (PK), which exists in two isoforms: the enzymatically active PKM1 and inactive PKM2. Palsson-McDermott and colleagues found that LPS stimulation upregulates *PKM2* mRNA and protein levels by nearly 3-fold [50]. Importantly, they found that TLR4 signaling also promotes phosphorylation of PKM2 at tyrosine-105, which maintains the protein in its enzymatically-inactive monomeric/dimeric formation. They went on to show that monomeric/dimeric PKM2 complexes with HIF-1 α in the nucleus and directly binds the IL-1 β promoter. Pharmacologic induction of the enzymatic activity of PKM2 prevents its interaction with HIF-1 α and ultimately polarizes macrophages towards an anti-inflammatory phenotype.

In addition, HIF- α -mediated glycolysis generates more pyruvate to enter the TCA cycle, which increases cellular concentration of TCA intermediates and the activity of TCA cycle enzymes. Mills *et al.* found that increased succinate dehydrogenase (SDH) activity raises the mitochondrial membrane potential, thus driving production of mitochondrial ROS and decreasing ATP production by the electron transport chain [44]. As such, it was shown that blockade of SDH decreases HIF-1 α activity and induces an anti-inflammatory phenotype in macrophages. Infantino *et al.* found that accumulation of citrate, another TCA intermediate, increases production of ROS, which have been shown to stabilize HIF-1 α and inhibit FIH-1 [51–53]. They also showed that proinflammatory macrophages activated by LPS or IFN- γ express elevated levels of the mitochondrial citrate carrier (CIC) [51]. CIC-mediated export of citrate to the cytoplasm is required for production of nitric oxide (NO) and prostaglandins in macrophages. While no work has shown a direct link between CIC and HIF-1 γ , one could speculate that exported citrate in proinflammatory macrophages promotes HIF-1 α activity. Together, these findings suggest that LPS stimulation induces the accumulation of succinate and citrate, both of which stabilize HIF-1 α either directly or indirectly [54]. The upregulation of glycolysis by HIF-1 α and subsequent stabilization and activation of HIF by glycolytic enzymes and TCA intermediates creates a positive feedback loop that drives macrophages to secrete IL-1 β , produce ROS, and kill invading microbes.

HIF-1 α activity is also crucial for the recruitment and antimicrobial function of macrophages. Over 15 years ago, Cramer *et al.* demonstrated that conditional deletion of HIF-1 α in the myeloid lineage does not impair development of monocytes and macrophages but hinders their migration and function [55]. Indeed, they showed that conditional myeloid HIF-1 α knockout mice are more susceptible to several bacterial infections [55]. Multiple groups have shown that the presence of hypoxia alone leads to a HIF-1 α -dependent upregulation of TLR protein and mRNA in macrophages that primes the cells to respond to

pathogen or damage signals [16,56]. Recruitment of monocytes to sites of infection or damage requires tracking and adhesion to local endothelial cells. Kong *et al.* observed that hypoxia causes a dramatic increase in adhesion of U937 monocytes to endothelial cells [57]. They went on to show that the promoter of the gene for integrin CD18 contains an HRE, directly linking HIF-1 α activity to monocyte adhesion and recruitment. We have recently demonstrated that HIF-1 α -deficient macrophages have hindered phagocytic capacity *in vitro* [58]. In addition, Anand and colleagues found that hypoxia or genetic overexpression of HIF-1 α enhances phagocytic capacity of activated macrophages, partially by promoting the p38-MAP kinase signaling pathway [59]. More recently, Braverman and Stanley showed that inducible nitric oxide synthase (iNOS) activates HIF-1 α in a NO-dependent manner to form a positive feedback loop amplifying antimicrobial activity [60]. During infection with *Mycobacterium tuberculosis*, which establishes latency in macrophages, NO was previously thought to have bactericidal activity against the infection. Instead, they found that NO acts as a signaling molecule downstream of IFN- γ to stabilize HIF-1 α and promote *M. tuberculosis* killing indirectly [60].

Macrophages also activate the adaptive immune system by presenting antigens on MHC molecules. Bhandari *et al.* found that HIF-1 α -deficient macrophages present fewer antigens via MHC class II and express lower levels of T cell co-stimulatory molecules CD80 and CD86 after stimulation with ovalbumin than their wildtype counterparts [61]. They further demonstrated the functional importance of HIF-1 α for vaccine-mediated T cell responses by showing that myeloid-specific HIF-1 α -deficient mice mount a weaker titer response than wildtype mice, while wildtype mice given a pharmacologic agonist of HIF-1 α activity mount an enhanced titer response. Together, these results demonstrate that HIF-1 α lies at the intersection of macrophage function and metabolism. Less is known about the extent to which the metabolic activity of HIF-1 α directly impacts its effects on antimicrobial function, although this is an active area of research in our lab and others.

One of the most striking discoveries regarding the role of HIF-1 α in macrophage function has been the observation that priming macrophages with microbial products that stimulate HIF-1 α activity leads to a durable alteration in macrophage phenotype that is sustained for up to one week [62–64]. We recently reported that the TLR4 agonist monophosphoryl lipid A (MPLA) induces a sustained metabolic program in macrophages initially characterized by elevated glycolysis and glutamine flux into the TCA cycle [64]. Three days after the removal of MPLA, macrophages retained elevated glycolysis, but also possessed enhanced oxidative phosphorylation and malate shuttling into the mitochondria, replenishing TCA cycle intermediates.

Remarkably, mice primed with MPLA were broadly protected against systemic infection with *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* [64,65]. We further showed that HIF-1 α -deficient macrophages are unable to mount this metabolic program in response to MPLA, and blockade of the mechanistic (formerly mammalian) target of rapamycin (mTOR), which stabilizes HIF-1 α , blocks the protective phenotype *in vivo* [58,64]. This process of innate immune “training” has been likened to immune memory in adaptive lymphocytes, albeit shorter lived and nonspecific [66].

Netea and colleagues showed that primed monocytes activate HIF-1 α , which leads to elevated glycolysis even in the presence of oxygen (aerobic glycolysis) and extensive epigenetic remodeling that sustains phenotypic changes in macrophages derived from these cells [66]. Furthermore, they corroborated the finding that mice primed with microbial products (in this case, β -glucan from fungal cell walls) are protected from a lethal intravenous *Staphylococcus aureus* infection [62]. Interestingly, the same group recently demonstrated that activation of cholesterol synthesis leads to an accumulation of mevalonate, which signals to insulin-like growth factor 1 receptor in an autocrine fashion, leading to HIF-1 α stabilization, elevated glycolysis, and trained macrophages [67]. Patients with hyper immunoglobulin D syndrome, who accumulate mevalonate due to mevalonate kinase deficiency, have monocytes with constitutively trained phenotypes. Subsequently, the process of trained innate immunity has been shown in malarial, fungal, and tuberculosis infections [68–70].

Several questions still remain regarding the role of HIF-1 α in macrophages. Mills and colleagues found that loss of HIF-1 α signaling prompts IL-10 production in macrophages, thus crafting an anti-inflammatory environment [44]. However, the signals that initially suppress HIF-1 α in anti-inflammatory macrophages are unknown. Ip *et al.* presented a clue by showing that IL-10 dampens LPS-induced glycolytic flux by preventing translocation of GLUT1 from intracellular vesicles to the cell surface and restricting mRNA synthesis of several genes involved in glycolysis [71]. This is mediated in part by inhibition of mTOR signaling, which is upstream of HIF-1 α . Even though these preliminary results are intriguing, it remains unclear whether production of IL-10 or inhibition of HIF-1 α occurs first and what signaling events connect the two phenomena.

Additionally, our lab and others have demonstrated that HIF-1 α activity is essential for macrophage recruitment and phagocytosis, yet the downstream targets of HIF-1 α that contribute to this phenotype are largely unknown. It has been hypothesized that enhanced glycolysis provides biosynthetic building blocks through the PPP to support antimicrobial function [72]. Others have proposed that inflammatory genes contain HREs that trigger robust mRNA expression following HIF-1 α stabilization [56]. It is likely that these mechanisms are contributing to macrophage inflammation, but more work is needed to parse out the details.

HIF-1 α Regulation in Neutrophils

Neutrophils are the most abundant and rapid responders to pathogens or damage signals. Neutrophils contribute to the maintenance of the inflammatory niche by releasing lytic enzymes from granules and generating ROS to aid in microbial killing [73]. While comparatively less work has studied the role of HIF-1 α in neutrophils, a mounting body of evidence suggest that hypoxia and HIF-1 α are part of a critical regulatory pathway in neutrophil survival and function [74]. HIF-1 α protein is detectable only at low levels in unstimulated neutrophils despite an abundance of *HIF1A* mRNA, suggesting that HIF-1 α activity is regulated by PHD-mediated post-translational destruction, similarly to other cells [75]. Inactivated neutrophils lie in a dormant-like state in the oxygen-rich bloodstream, which minimizes HIF-1 α activity. When they extravasate to sites of inflammation, exposure

to the hypoxic environment triggers HIF-1 α stabilization and subsequent neutrophil activation [74].

Activated neutrophils typically undergo apoptosis within 72 hours of stimulation [76,77]. This apoptotic response is beneficial for limiting inflammatory damage to host tissues. However, HIF-1 α activation limits neutrophil apoptosis during hypoxia. [78]. Over a decade ago, Walmsley and colleagues discovered that HIF-1 α ablation significantly hinders neutrophil survival under hypoxia. Furthermore, HIF-1 α activity triggers NF- κ B stabilization and macrophage inflammatory protein-1 β (MIP-1 β) secretion, both of which also promote neutrophil survival [79]. This phenomenon is similar to what is seen in macrophages, where HIF-1 α transcription is enhanced by NF- κ B in macrophages. However, macrophage HIF-1 α activity may be promoted by I κ B, an NF- κ B inhibitor, due to interactions with with FIH-1 [27,34]. Given these seemingly conflicting results in macrophages, the interaction between HIF-1 α and NF- κ B in neutrophils should be further studied.

Several regulatory mechanisms promote neutrophil apoptosis by reducing HIF-1 α activity. One such mechanism involves the accumulation of intracellular ascorbate. Vissers and Wilkie showed that mice deficient in L-gulonolactone oxidase (*Gulo*^{-/-}), which are unable to synthesize ascorbate, produce neutrophils with constitutively elevated HIF-1 α activity [80]. Peritoneal neutrophils stimulated by thioglycolate in *Gulo*^{-/-} mice demonstrated enhanced survival after 18 hours as measured by exposure of phosphatidylserine and propidium iodide/annexin V staining. Furthermore, macrophage phagocytosis of aged neutrophils in *Gulo*^{-/-} mice was decreased compared to wildtype cells. Because ascorbic acid is a required cofactor for PHD enzymatic activity, the authors hypothesized that intracellular ascorbate accumulation optimizes PHD-mediated HIF-1 α degradation in wildtype neutrophils. This hypothesis is supported by the observation that mature peripheral blood neutrophils contain high levels of intracellular ascorbate [81]. Additionally, Walmsley and colleagues found in another study that neutrophils from patients with heterozygous loss-of-function mutations in pVHL exhibit delayed apoptosis under normoxic conditions and enhanced bacterial phagocytic capability [82]. Hypoxia further promoted survival in pVHL-deficient neutrophils, suggesting that HIF-1 α controls neutrophil apoptosis by several mechanisms.

Neutrophils are dependent on glycolysis to meet energy demands [83]. Several studies have shown that HIF-1 α activity alters glucose utilization in neutrophils, thereby dictating activation and function. Schuster *et al.* demonstrated that HIF-1 α is required for translocation of the glucose transporter GLUT1 to the surface of neutrophils after activation [24]. Furthermore, they demonstrated that increased glucose consumption is crucial for enhanced neutrophil survival as neutrophils enter hypoxia. Recently, Sadiku and colleagues showed that *Streptococcus pneumoniae* pneumonia in *Phd2*^{-/-} mice leads to exaggerated neutrophil influx into the alveolar space, with fewer cells undergoing apoptosis at 72 hours, compared to wildtype mice [84]. Neutrophils from *Phd2*^{-/-} mice also show enhanced chemotaxis in response to the chemokine KC (murine analog of human IL-8).

They went on to demonstrate that this response is due to increased neutrophil glycolytic capacity secondary to constitutive HIF-1 α expression. Neutrophils cultured *ex vivo* from infected *Phd2*^{-/-} mice display similar increases in chemotaxis and survival that can be completely abrogated in glucose-free media. Furthermore, intraperitoneal injection of the non-functional glucose analogue 2-deoxyglucose (2-DG) into *Phd2*^{-/-} mice significantly blunts lung inflammation after inhalation of nebulized LPS. Together, these studies suggest that HIF-1 α coordinates glucose metabolism to optimize neutrophil survival and function during acute inflammation.

HIF-1 α also plays a direct role in neutrophil antimicrobial activity. Neutrophil extracellular traps (NETs) are complex matrices of antimicrobial proteins, DNA, and histones that ensnare and lyse microbes [85]. McInturff *et al.* found that HIF-1 α is essential for deployment of NETs by human neutrophils stimulated with LPS [86]. In response to LPS, neutrophils activate mTOR, which post-translationally stabilizes HIF-1 α and subsequently promotes NET formation. Inhibition of either mTOR or HIF-1 α activity was found to be sufficient to abrogate NET release and hinder neutrophil bacterial killing *in vitro*. Additionally, Vollger and colleagues demonstrated that direct stabilization of HIF-1 α by deferroxamine alone is sufficient to induce NET formation in neutrophils *ex vivo* [87]. Finally, Rodriguez-Espinosa and colleagues found that NET formation and release depends on glycolysis, a process heavily regulated by HIF-1 α [88]. Furthermore, they found that treatment of neutrophils with 2-DG or removal of glucose from media blocks NET release after LPS stimulation. The importance of HIF-1 α for neutrophil antimicrobial function was further demonstrated by Elks *et al.* during zebrafish *Mycobacterium marinum* infection, which closely replicates human tuberculosis [89]. Both pharmacologic and genetic stabilization of HIF-1 α early in the infection promotes bacterial clearance by increasing reactive nitrogen species (RNS) in neutrophils that remove larval mycobacteria. They concluded that increased HIF-1 α activity drives RNS formation in part by promoting neutrophil iNOS [89–91].

In contrast to acute hypoxia, preconditioning animals with prolonged hypoxia reduces neutrophil glycolytic metabolism and recruitment during bacterial infections with *Staphylococcus aureus* and *Streptococcus pneumoniae* [92]. Additionally, neutrophil HIF-1 α activity is diminished for several days following hypoxic preconditioning. Restriction of acute inflammation by hypoxic preconditioning leads to preservation of body temperature and decrease in sickness score during infection. Furthermore, preconditioned mice have improved cardiac function and prolonged survival as compared to hypoxia-naïve mice. The results of hypoxic preconditioning on infection outcomes could be replicated by myeloid-specific HIF-1 α knockout in hypoxia-naïve mice. Together the studies described above demonstrate mechanisms to maintain the fine balance of HIF-1 α activity that must be struck to enable appropriate neutrophilic inflammation to clear invading pathogens while avoiding host injury.

Research into the signaling mechanisms coupling HIF-1 α activity with neutrophil survival and function is lacking. It still remains unknown which HRE-containing genes are preferentially activated in neutrophils during hypoxia and inflammation. Additionally, a more sophisticated assessment of neutrophil metabolism, and the importance of HIF-1 α

therein, will strengthen our understanding of the connections between myeloid metabolism and function. For example, Azevedo *et al.* recently expanded upon the finding that NET formation requires glycolysis by showing that increased flux of glucose-6-phosphate from glycolysis into the PPP is essential for NET formation and release [93]. However, it is unclear if genes induced by HIF-1 α modulate the PPP, or any other metabolic pathways outside of glycolysis, in neutrophils.

A major limitation of the work in this field is the lack of neutrophil-specific HIF-1 α deletion strategies. Most genetic deletions in mice or zebrafish models ablate HIF-1 α in the entire myeloid compartment. Given the essentiality of HIF-1 α for macrophage and dendritic cell function, these models suffer from a lack of resolution on neutrophils. To combat this, studies have focused on neutrophil-centric models of inflammation, but to tease apart the importance of neutrophil HIF-1 α activity broadly, more sophistication is required. One opportunity lies in the use of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 to target neutrophils in zebrafish [94,95]. Additionally, mice with Cre recombinase expressed at the Ly6G promoter (neutrophil specific) could generate mice with neutrophil-specific HIF-1 α deletion for infection studies [96].

HIF-1 α Regulation in Dendritic Cells

Myeloid-derived dendritic cells (DCs) are responsible for sampling antigens in their environment to detect potential pathogens. Once a DC detects a microbial pattern, it migrates to a regional draining lymph node (LN) to present antigens to T cells. This process initiates DC maturation, which is characterized by increased antigen presentation capability, cytokine secretion, and morphologic changes [97]. DC migration from peripheral tissue to LNs is dependent upon a chemokine gradient detected primarily by C-C chemokine receptor type 7 (CCR7) on the surface of DCs. Kohler *et al.* demonstrated that immature DCs exposed to hypoxia upregulate CCR7 in a HIF-1 α dependent fashion [98]. Bone-marrow derived DCs from HIF-1 α -deficient mice failed to express CCR7 almost entirely while CCR5, which recruits DCs to sites of inflammation, was unaffected [98]. Ricciardi *et al.* recapitulated these results, showing that monocyte-derived immature DCs grown in hypoxia developed a strong migratory phenotype while dampening proinflammatory cytokine secretion [99]. Guak and colleagues linked this phenomenon to metabolism by demonstrating that both strong and weak stimuli will induce glycolysis in DCs, but only strongly proinflammatory signals will stabilize HIF-1 α , leading to a prolonged reprogramming of DC metabolism towards glycolysis [100]. Furthermore, Jantsch *et al.* showed that immature DCs stimulated with LPS under hypoxia, compared to normoxia, have a dramatic increase in T cell costimulatory molecule expression that leads to enhanced lymphocyte proliferation [101]. Together these studies demonstrate that immature DCs exposed to hypoxia, as in the inflammatory niche, develop a phenotype optimized to rapidly migrate and present antigen to cells of the adaptive immune system. When DCs are in normoxic conditions, TLR stimulation alone leads to HIF-1 α activation. Siegert *et al.* found that TLR-mediated NF- κ B activation decreases intracellular iron stores in DCs by induction of ferritin, which sequesters bioavailable iron [102]. In turn, PHD activity was diminished, as iron is a critical co-factor for PHD enzymatic activity along with oxygen [102].

Several studies have explored how HIF-1 α controls DC maturation, metabolism, and, ultimately, their subsequent adaptive immune activation [103]. Krawczyk and colleagues found that immediately after TLR stimulation, DCs increase glucose consumption and GLUT1 expression by activating phosphoinositide 3-kinase (PI3K) signaling, which directly leads to HIF-1 α stabilization [62,104]. Moreover, they found that inhibition of glycolysis diminishes the capacity of DCs to respond to TLR activation [101,104]. Everts *et al.* found that achieving this rapid surge in glycolytic metabolism requires DCs to activate iNOS, thereby increasing levels of NO, which inhibits mitochondrial oxygen consumption [105]. As described above, NO rapidly stabilizes HIF-1 α , which also increases NO production, creating a positive feedback loop. Thus, it is likely that HIF-1 α activation continues to play an important role in inflammatory DC activity, even after the initial stimulus [60]. Corroborating this, Perrin-Cocon and colleagues recently found that inhibition of HIF-1 α with echinomycin prevents secretion of proinflammatory cytokines by DCs [106]. Using a library of drugs to screen for upstream mediators of this effect, the authors found that inhibition of p38-MAP kinase recapitulated the effect of HIF-1 α inhibition. This is interesting given that p38-MAP kinase signaling was also shown to be required for HIF-1 α mediated enhancement of macrophage phagocytosis [59]. This similarity between the two cell types should prompt further investigation into the signaling mechanisms that mediate enhancement of antimicrobial activity by HIF-1 α .

Based on the nature of the local cytokine milieu and signals from DCs, CD4⁺ T cells differentiate into type 1 (Th1) and type 2 (Th2) helper cells that tailor adaptive responses against intracellular pathogens (bacteria, fungi) and helminths, respectively. Xiong *et al.* showed that HIF-1 α activity in DCs influences T helper fate determination by stimulating surface expression of Jagged2, in part by limiting Krüppel-like factor (KLF)-2 signaling [107]. Increased surface Jagged2 binds to Notch receptors on naïve CD4⁺ T cells, promoting their Th2 polarization by driving IL-4 secretion. It was further demonstrated that HIF-1 α promotes Th2 polarization by Hammami *et al.*, who found that HIF-1 α inhibits IL-12 production in DCs, thereby limiting Th1 development [108]. Contrary to these findings, Liu *et al.* showed that DCs lacking SIRT1, which enhances HIF-1 α degradation by deacetylation, promote IL-12 production after LPS stimulation by stabilization of HIF-1 α [109]. KLF and SIRT1 have targets other than HIF-1 α , which may partly explain these conflicting results. However, these results also highlight the heterogeneity and specificity of HIF-1 α binding. Further studies utilizing genomic approaches to determine how HIF-1 α directs the expression of certain genes are required to provide more mechanistic insight to these questions.

In chronic autoimmune conditions, DCs have been found to promote expansion of regulatory T cells (Tregs), which limits immunologic damage to host tissue. For example, in the gut, where constant pathogen interaction occurs, Tregs prevent development of colitis. The loss of this anti-inflammatory mechanism has been shown to play a significant role in inflammatory bowel disease (IBD) [110]. Sun *et al.* found that DCs in the lamina propria of the small intestine drive the maturation of Tregs from CD4⁺ T helper cells [111]. Flück and colleagues demonstrated that in mice lacking DC HIF-1 α activity, dextran sodium sulfate (DSS) colitis (a model of IBD) was significantly more severe, in part due to a lack of maturation of Tregs and increased IL-6 and IL-23 secretion [112]. These results also suggest

that HIF-1 α activity preferentially promotes location- and organ-specific DC functions. Future studies should continue to dissect the varied roles of DCs and their regulation by HIF-1 α , as any potential therapeutic targeting of DC HIF-1 α activity will require a nuanced understanding of tissue-specific side effects.

Myeloid HIF-1 α Activity in Atherosclerosis

Inappropriate hypoxic signaling in myeloid cells can also contribute to pathologic states. The best characterized of these pathologies is the development of atherosclerotic lesions. Macrophages are attracted to developing atherosclerotic lesions and phagocytose oxidized low-density lipoprotein (oxLDL), adopting a foam cell morphology that contributes to growth and instability of the plaque [113]. Conversely, inhibition of HIF-1 α by siRNA prevents oxLDL induction of foam cells in U937 monocytes *in vitro* [114]. Multiple groups have found that HIF-1 α expression is higher in foam-cell macrophages and likely plays a role in the pathogenesis of the disease [115–117]. Parathath *et al.* described that macrophages in hypoxic areas of atherosclerotic plaques contain elevated levels of cholesterol due to decreased expression of the cholesterol efflux protein ATP-binding cassette subfamily A member 1 (ABCA1) [118]. Knockdown of HIF-1 α with short hairpin RNA in hypoxic macrophages improves cholesterol efflux through ABCA1 and normalizes cellular sterol levels. Furthermore, Folco *et al.* discovered that in human atherosclerotic plaques, HIF-1 α expression colocalizes with macrophage IL-1 α and caspase-1 activity, suggesting that hypoxia triggers inflammatory activation and potential disruption of the plaque [119].

Macrophages further contribute to the pathophysiology of atherosclerosis by promoting retention of apoB-100 lipoproteins in the vasculature intima at sites of potential plaque formation [120]. Macrophages synthesize and secrete negatively charged glycosaminoglycans (GAGs), such as versican, into the vasculature, which attract the positively charged lipoproteins. Asplund and colleagues showed that hypoxic macrophages secrete longer and more sulfated GAGs which bind and retain LDL particles with higher affinity [121]. Using genetic knockdown of HIF-1 α in cultured THP-1 monocytes, the same group demonstrated that macrophage versican synthesis and secretion was partially dependent on HIF-1 α activity [122]. Aarup *et al.* found that in a mouse model of atherosclerosis, bone marrow transplantation with *HIF-1 α ^{-/-}* myeloid cells dramatically reduces atherosclerotic burden [123]. Additionally, adipose tissue contains high concentrations of both monocyte-derived and resident macrophages that drive inflammation and adiposity in a HIF-1 α -dependent manner [124,125]. Finally, Marsch and colleagues found that repeated exposure of atherosclerosis-prone mice to 95% oxygen normalizes hypoxia in atherosclerotic plaques and ultimately shrinks the size of the necrotic core due to enhanced efferocytosis of necrotic cells by macrophages [126]. Taken together, these results suggest that HIF-1 α is an attractive therapeutic target to ease the burden of atherosclerosis. However, more work is needed to clearly define the role of hypoxia and HIF-1 α in atherosclerosis and to design targeted therapeutic delivery strategies to atherosclerotic plaques.

Discussion and Future Perspectives

Numerous redundancies within hypoxic and inflammatory signaling pathways ensure that myeloid cells recognize, kill, and clear invading pathogens in the inflammatory niche. As a responder to both hypoxia and inflammatory signals, HIF-1 α is tasked with integrating diverse environmental signals and coordinating a transcriptional response that facilitates optimal function in each myeloid cell. It is now clear that a vast array of myeloid antimicrobial functions depends on HIF-1 α activity (Figure 2). While inflammation is an essential element of the host response to infection, the establishment of chronic inflammatory niches contributes to a variety of human diseases.

The development of strategies to target myeloid HIF-1 α signaling offers promise to have unprecedented control over the inflammatory response and should be a strong focus of future studies [127]. However, modulation of myeloid HIF-1 α activity as a strategy to combat disease has yet to be tested in human studies. While we currently lack myeloid-specific targeting strategies, and the effects of unrestrained hypoxic signaling in myeloid cells have not been fully explored, several lines of evidence suggest the feasibility of targeting HIF-1 α therapeutically. First, PHD inhibitors are currently being tested in phase II and III clinical trials for the treatment of anemia of chronic disease [128]. To date no adverse clinical outcomes have been observed with PHD inhibitors, although theoretical risks have been proposed [129]. Second, work from our laboratory has shown that priming mice with inflammatory stimuli leads to broad protection against Gram-positive and Gram-negative bacteria *in vivo* in a HIF-1 α -dependent manner [58,64]. In particular, the discovery of HIF-1 α -driven trained innate immunity provides both prophylactic and therapeutic options for patients with a high risk of infection, such as the critically ill. As trained immunity focuses on enhancing the host response to infection, rather than relying on antibiotics, it does not exacerbate antimicrobial resistance amongst infectious pathogens [130]. Surprisingly, pre-clinical models have demonstrated that global inhibition of PHD function delays development of atherosclerotic plaques due to altered hepatic cholesterol metabolism, which is in stark contrast to myeloid-specific HIF-1 α inhibition [131]. However, potential consequences due to myeloid HIF-1 α activity in these patients must be considered. These examples highlight the potential impact that a robust understanding of HIF-1 α activity in myeloid could have on human health. As we come to more fully understand the fundamental mechanisms by which HIF-1 α shapes the inflammatory response, a new focus should emerge with an eye toward translating these findings into clinical practice.

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Abbreviations

2-DG	2-Deoxyglucose
ABCA1	ATP-Binding Cassette Subfamily A Member 1
ATP	Adenosine Triphosphate

CCR	C-C Chemokine Receptor
CD	Cluster of Differentiation
CIC	Mitochondrial Citrate Carrier
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DCs	Dendritic Cells
DMOG	Dimethyloxallyl Glycine
DSS	Dextran Sodium Sulfate
FAO	Fatty Acid Oxidation
FIH-1	Protein Factor Inhibiting HIF-1
GLUT1	Glucose Transporter 1
Gulo	L-gulono- γ -lactone oxidase
HIF	Hypoxia-Inducible Factor
HREs	HIF Response Elements
IBD	Inflammatory Bowel Disease
IFN-γ	Interferon-Gamma
IKK-β	I κ b Kinase Beta
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
IκB	Inhibitor of Nuclear Factor-Kappa B
KLF-2	Krüppel-Like Factor 2
LDL	Low-Density Lipoprotein
LPS	Lipopolysaccharide
MHC	Major Histocompatibility Complex
MIP-1β	Macrophage Inflammatory Protein-1 β
MPLA	Monophosphoryl Lipid A
mTOR	Mechanistic Target Of Rapamycin
NETs	Neutrophil Extracellular Traps
NF-κB	Nuclear Factor of Kappa B
NO	Nitric Oxide

oxLDL	Oxidized Low-Density Lipoprotein
PHDs	Prolyl-4-Hydroxylase Domain Enzymes
PI3K	Phosphoinositide 3-Kinase
PK	Pyruvate Kinase
PKM	Pyruvate Kinase Muscle Isoenzyme
PPP	Pentose Phosphate Pathway
PVHL	Von Hippel-Lindau E3 Ubiquitin Ligase
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SDH	Succinate Dehydrogenase
SIRT1/2	Sirtuin Deacetylase 1 and
TCA cycle	Tricarboxylic Acid Cycle
Th1/2	T-Helper Cell Type 1 and 2
TLR	Toll-Like Receptor
Treg	Regulatory T Cell
VEGF	Vascular Endothelial Growth Factor

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Fig. 1. Regulation and Activation of HIF-1α in Macrophages.

During hypoxia and inflammation, HIF-1α is stabilized and complexes with HIF-1β. The complete HIF-1 complex translocates to the nucleus where it binds to HIF responsive elements (HREs) with the CREB-binding protein (CRBP)/p300 coactivators. Genes with HREs in their promoters include metabolic enzymes and proinflammatory mediators. Under normal oxygen tension, HIF-1α is degraded in the cytoplasm due to oxidation of proline residues by prolyl-4-hydroxylase domain enzymes (PHDs) in an oxygen-dependent manner. Prolyl hydroxylation recruits the von Hippel-Lindau E3 ubiquitin ligase (pVHL), which ubiquitinates and targets HIF-1α for destruction by the proteasome. This process is enhanced by sirtuin (SIRT) 1 and 2, which deacetylate lysine residues on HIF-1α, in turn enhancing PHD affinity. HIF-1α activity increases surface expression of glucose transporter 1 (GLUT1) and activity of glycolytic enzymes. Pyruvate kinase isoenzyme M2 (PKM2), which catalyzes the conversion of phosphoenolpyruvate to pyruvate, can translocate to the nucleus with HIF-1 and directly bind promoters of proinflammatory genes. Additionally,

HIF-1 α stimulates increased anaplerotic flux of glutamine into the TCA cycle. Together, glucose and glutamine drive activity of succinate dehydrogenase (SDH) and accumulation of cytosolic succinate. An increase in the succinate/ α -ketoglutarate (α -KG) ratio directly promotes HIF-1 α stabilization and prevents its degradation.

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Fig. 2. Summary of Major Roles for HIF-1α Activity in Myeloid Cells.

A common myeloid progenitor cell gives rise to several myeloid cells including neutrophils, monocytes, macrophages, and dendritic cells. These myeloid cells comprise most of the innate immune system. HIF-1α is essential for the activation and antimicrobial functions of each of these cell types. HIF-1α universally enhances glycolysis. Additionally, it is essential for core cellular functions such as neutrophil extracellular trap (NET) formation in neutrophils, phagocytosis in macrophages, and antigen presentation by dendritic cells.