The Regulation of Pulmonary Inflammation by the Hypoxia-Inducible Factor–Hydroxylase Oxygen-Sensing Pathway

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Abstract

Although the hypoxia-inducible factor (HIF)–hydroxylase oxygen-sensing pathway has been extensively reviewed in the context of cellular responses to hypoxia and cancer biology, its importance in regulating innate immune biology is less well described. In this review, we focus on the role of the HIF-hydroxylase pathway in regulating myeloid cell responses and its relevance to inflammatory

lung disease. The more specific roles of individual HIF/ prolyl hydroxylase pathway members *in vivo* are discussed in the context of lineage-specific rodent models of inflammation, with final reference made to the therapeutic challenges of targeting the HIF/ hydroxylase pathway in immune cells.

Keywords: inflammation; hypoxia inducible factor; hydroxylase; neutrophil; macrophage

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The Hypoxia-Inducible Factor–Hydroxylase Oxygen-Sensing Pathway

The identification of hypoxia-inducible factor (HIF) has proved of fundamental importance, opening up the biology of a pathway that allows cells and organisms to adapt to both acute and chronic changes in oxygen tension (1). HIF is a transcription factor that regulates the expression of many hundreds of genes and is itself tightly regulated by oxygen availability (2, 3). HIF is expressed by almost all cell types, and the components of the oxygen-sensing pathway are widely conserved across evolution (4, 5).

HIF proteins are heterodimers, composed of a constitutively expressed β subunit together with an α subunit that is regulated both at a transcriptional level (6) and by the regulation of protein expression by ubiquitination. In the presence of oxygen, HIF- α subunits are hydroxylated at two proline residues by the prolyl hydroxylase (PHD) family of enzymes,

PHDs1–3 (reviewed in [7]). Initially, PHD2 was viewed as the primary PHD regulating HIF hydroxylation in normoxia, although specific roles for other PHDs have also emerged (8, 9). The PHDs are the “oxygen-sensing enzymes” and are inactivated at reduced oxygen concentrations, allowing stabilization of HIF protein (10, 11).

Although prolyl hydroxylation, targeting HIF- α for degradation via the von Hippel Lindau E3 ubiquitin ligase (VHL) (11), is the principal regulator of HIF activity, a further layer of regulation is that HIF transactivation can be inhibited by asparaginyl hydroxylation via factor inhibiting HIF (FIH) (12). Sustained HIF expression can in turn result in a negative feedback loop, with HIF-mediated transcription of PHDs and VHL having the potential to down-regulate expression of HIFs. The PHDs, FIH, VHL, HIF-1 α , and HIF-2 α , a closely related HIF- α subunit (13), are thus viewed as the main players in the oxygen-sensing pathway.

The initial focus of research on this pathway has been on the regulation of

cellular responses to hypoxia, but it is increasingly recognized that cytokines and other proinflammatory stimuli such as bacteria and bacterial LPS can regulate the transcription of HIFs, allowing synergistic effects when combined with stabilization of HIF proteins by coexistent hypoxia. The promoter of the HIF-1 α gene has a nuclear factor (NF)- κ B binding site and both basal and induced HIF-1 α transcription are NF- κ B dependent, although this does not appear to be the case for HIF-2 α . Hypoxia can, in turn, augment NF- κ B expression, and this is impaired in HIF-1 α -deficient cells, demonstrating important cross-talk between the HIF and NF- κ B pathways that results in bidirectional amplification of signaling (14, 15).

The HIF-Hydroxylase Pathway and the Lung

The lung can experience hypoxia in pathological but sometimes also physiological situations (e.g., during

lung development *in utero* or residence at high altitude), with associated alveolar hypoxia. The HIF-hydroxylase pathway has been implicated in diverse lung pathologies associated with hypoxia that may be localized (e.g., airway obstruction secondary to mucus plugging) or generalized, as in the more diffuse alveolar hypoxia associated with acute lung injury or even COPD. The roles of the HIF-hydroxylase pathway in the development of pulmonary hypertension and hypoxic vasoconstriction have also been extensively studied (16). This review, however, focuses on the roles of HIF pathway components in regulating myeloid cell responses to lung infection and inflammation, in particular how this pathway regulates the acute inflammatory responses of neutrophils and tissue macrophages.

The HIF-Hydroxylase Pathway and Innate Immune Cell Biology

Circulating immune cells, including neutrophils and monocytes, typically encounter a range of oxygen tensions, with oxygen availability reducing as cells migrate into tissues and reducing further in inflamed as opposed to healthy tissue. The profound hypoxia typical of inflamed tissues results from a combination of reduced oxygen availability and increased oxygen consumption by inflammatory cells entering the tissue (17). HIFs regulate a number of important cellular functions in these cells, particularly cellular energetics, up-regulating glycolytic enzymes and glucose transporters to permit ATP generation under conditions of hypoxia (1). This metabolic adaptation to hypoxia underpins the ability of immune cells, particularly neutrophils, to remain viable in hypoxic tissues. Indeed, hypoxia has a prosurvival effect on both neutrophils and monocytes (18, 19), extending their lifespan in an HIF-1-dependent manner (20, 21), via the inhibition of their spontaneous apoptosis.

Hypoxia, the HIF-Hydroxylase Pathway, and Neutrophil Biology

Neutrophils would appear intrinsically well adapted to function under conditions of reduced oxygen availability, with a dependence on anaerobic glycolysis for

ATP generation even when oxygen supply is plentiful (22, 23). Furthermore, in addition to extending cellular lifespan, hypoxia has been shown to up-regulate a number of proinflammatory functions in neutrophils. Hypoxia increases neutrophil expression of antimicrobial peptides and elastase release and also promotes the formation of extracellular traps (24–26). Studies of HIF-1-deficient murine neutrophils have demonstrated the dependence of these phenotypes on HIF-1 expression (27). Other neutrophil functions, including chemotaxis and phagocytosis, are preserved but not increased in hypoxia (25). In contrast, one important functional defect in hypoxic neutrophils is impaired reactive oxygen species-dependent killing of bacteria. This is particularly notable in the context of *Staphylococcus aureus* infections and may in part explain the ability of this organism to subvert the innate immune response, resulting in significant tissue destruction and abscess formation within hypoxic tissues (25).

Neutrophils also express HIF-2 α , which unlike HIF-1 α is expressed at a low level in unstimulated neutrophils but again up-regulated by hypoxia and by bacteria (28). HIF-2 deficiency does not impair neutrophil functions such as chemotaxis, phagocytosis, and respiratory burst. HIF-2 has fewer metabolic targets than HIF-1 and, possibly for this reason, does not modulate neutrophil lifespan *ex vivo*, although tissue neutrophils deficient in HIF-2 display increased sensitivity to nitrosative stress when studied *ex vivo* (28).

The roles of other HIF pathways members have yet to be extensively studied in neutrophils. Neutrophils derived from patients with heterozygous germline mutations in the VHL protein were found to have enhanced survival in normoxia, compatible with increased HIF expression, and also enhanced phagocytosis of bacteria (29). There is no published information on the roles of PHDs 1 and 2 in neutrophils, but studies of PHD-deficient neutrophils have revealed an unexpected phenotype in which PHD3 may act “downstream” of HIFs, having no effect on expression of HIF or HIF target genes but controlling expression of specific apoptosis-regulating genes, Bcl-x_L and SIVA-1, and thus regulating neutrophil survival in hypoxia (30).

Hypoxia, the HIF-Hydroxylase Pathway, and Monocytes-Macrophages

Macrophages also up-regulate HIF-1 α and HIF-2 α in hypoxia, with HIF-1 playing a major role in determining macrophage metabolic responses (31, 32). HIF-1 α -deficient macrophages have reduced expression of glycolytic pathway components and impaired glycolysis, whereas VHL-deficient macrophages, with stabilized HIF-1 α , have increased glycolysis and release more lactate, which is a product of the glycolytic pathway (20, 31). Intermediates of cellular metabolism can in turn influence HIF pathway activity, with PHD enzymes being inhibited by intermediate molecules in the tricarboxylic acid cycle, which are generated during aerobic metabolism (7). One such intermediate, succinate, was recently shown to inhibit PHDs in macrophages, resulting in HIF-1 α stabilization and, importantly, enhancing IL-1 β release (33). Hypoxia enhances the phagocytic efficiency of macrophages both for bacteria and for apoptotic cells, effects that are abrogated by siRNA to HIF-1 α .

Expression of both opsonin receptors and scavenger receptors is increased, and intracellular killing of bacteria is also enhanced by HIF-1 α , potentially via HIF effects on inducible nitric oxide synthase (iNOS) expression (34–36). HIF-1 α also regulates macrophage production of proinflammatory cytokines, including tumor necrosis factor- α and IL-1 β , and of the proangiogenic factor vascular endothelial growth factor (20, 24). HIF-2 α also has important proinflammatory functions, because myeloid-specific *Hif2a* deletion inhibits *in vitro* secretion of proinflammatory cytokines IL-1 β , IL-6, and IL-12 in response to a combined IFN- γ /LPS stimulus (37). HIFs can have important indirect immunomodulatory roles, for example HIF-1 α mediating the effects of transforming growth factor- β 1 on profibrotic gene expression by alveolar macrophages (38). The distinct and shared immunomodulatory functions of HIF-1 α and HIF-2 α are areas of active investigation. Although structurally very similar, HIF-1 α is more widely expressed and more rapidly up-regulated (e.g., after intrapulmonary LPS challenge) but also has a shorter half-life than HIF-2 α protein (26, 36). Although many of their gene targets are shared, others, such as the

adenosine receptor A2aR, and a number of metabolic genes, are regulated only by a single HIF (31, 39).

Relative expression of HIF-1 and HIF-2 may also be an important marker, and potentially a driver, of macrophage polarization. HIF-1 α mRNA and protein are induced during M1 polarization of macrophages, whereas HIF-2 α is induced during M2 polarization and is decreased by stimulation with M1 polarizing factors (LPS/IFN- γ) (40). The relative levels of HIF-1 α and HIF-2 α may play an important role in regulation of nitric oxide (NO) metabolism by controlling levels of both iNOS and arginase (which competes with iNOS for the L-arginine substrate required for NO production). *Hif2 α ^{-/-}* macrophages exhibit normal hypoxic induction of iNOS (*Nos2*), whereas basal expression and hypoxic induction of arginase 1 (*Arg1*) is attenuated. Conversely, *Hif1 α* deletion dramatically reduces hypoxic iNOS induction, with a smaller effect on arginase expression (37, 40).

The roles of PHD enzymes in macrophages have also been studied. PHD3-deficient macrophages, unlike neutrophils, show increased HIF-1 α expression, enhanced cytokine release, and enhanced phagocytosis of both opsonized zymosan and apoptotic cells (41). Macrophages with heterozygous deficiency in PHD2 show a bias toward an M2 phenotype, with higher expression of M2-associated genes, including *Arg1* (42). These data may suggest that, at least in this cell type, PHD2 dominantly suppresses HIF-2 expression, whereas PHD3 has a greater role in regulating HIF-1.

Roles of HIF-Hydroxylase Pathway Components in *In Vivo* Models of Inflammation

Information on the *in vivo* functions of HIF pathway components has derived largely from mice deficient in pathway components, often cell-type restricted, but also from zebrafish models of inflammation that permit direct visualization of immune cell behavior (43) (Table 1). In zebrafish, increased expression of either HIF-1 or HIF-2 in the whole organism does not modify neutrophil recruitment but does delay the resolution of neutrophilic inflammation in response to tail-fin injury. The phenotype of delayed inflammation

resolution is due both to delayed neutrophil apoptosis and to retention of neutrophils at the site of injury, as demonstrated by delayed retrograde movement (28, 44). Early expression of HIF-1 α in zebrafish does, however, confer host protection from mycobacterial infection, decreasing bacterial numbers in an iNOS-dependent manner (45).

Myeloid-cell-specific deficiency of HIF-1 in mice (using the LysMcre promoter) demonstrated impaired innate responses in HIF-1-deficient neutrophils and macrophages, as discussed above, with reduced inflammation in skin and joint disease models (20). Mice with myeloid deficiency of HIF-2 similarly show reduced inflammation in *in vivo* models of cutaneous and peritoneal inflammation and, in particular, reduced cytokine production (37).

A potentially important difference between HIF-1 and HIF-2 is the profound phenotype of HIF-1 deficiency in the context of infection. Mice with myeloid-specific HIF-1 deficiency showed inability to control Group A *Streptococcus* infection, both locally and systemically, in keeping with their *in vitro* phenotype of impaired bacterial phagocytosis and killing (24). Conversely, treatment with HIF agonists (e.g., mimosine) can improve bacterial clearance (46), and enhanced HIF-1 expression improved host responses to mycobacterial infection as above (45). These data, in combination, suggest targeting HIF-2 is a more plausible antiinflammatory therapeutic strategy, because its inhibition would result in less impairment of antimicrobial responses.

Studies in mice deficient in PHD enzymes have also generated interesting *in vivo* phenotypes. Haploinsufficiency of PHD2 in myeloid cells skews macrophages to an M2 phenotype distal to femoral artery ligation, increasing release of arteriogenic factors (42). In a systemic sepsis model, however, the same strain of mice showed decreased survival due to an overwhelming systemic inflammatory response syndrome (SIRS) response (41). The effects of PHDs 1 and 2 on acute inflammation remain to be elucidated.

The HIF-Hydroxylase Pathway in Pulmonary Inflammation

Observational studies of humans living at high altitudes have identified, in the context

of systemic hypoxia, higher levels of circulating inflammatory mediators (47). In rodent models of systemic hypoxia, moreover, a basal increase in alveolar macrophage numbers is observed (48). Acutely, in the context of acute lung injury, hypoxia has been shown to increase the release of CXCL-8, an important neutrophil-recruiting chemokine, by alveolar macrophages (49). In a mouse model of ischemia-reperfusion injury, whole-animal HIF-1 heterozygotes were protected from a SIRS response in the lung and showed reduced cytokine production, in keeping with a proinflammatory role for HIF-1 *in vivo* (50). Together, these studies provide supportive evidence for the role of systemic hypoxia and HIF-1 in activating innate immune responses in the lung. Conversely, an important immunomodulatory role for HIF-1 α was recently demonstrated in the lung. After allergen exposure in mice, macrophage deficiency of HIF-1 resulted in impaired IL-10 secretion and thus in increased dendritic cell activation and stimulation of Th2 responses, resulting in increased allergic airway inflammation (51).

In mice with myeloid-specific deficiency of HIF-2, in a model of LPS-induced acute lung injury, there is accelerated clearance of HIF-2-deficient neutrophils from the lungs and enhanced neutrophil apoptosis, perhaps due to enhanced susceptibility to nitrosative stress (28), and this is associated with evidence of reduced lung injury. In keeping with expression patterns of HIF-2 in human and murine myeloid cells, this phenotype was present in normoxic mice.

PHD-deficient mice display a hypoxia-specific phenotype in the context of neutrophilic inflammation in the lungs, with myeloid-specific deficiency of PHD3 leading to reduced neutrophilic inflammation, due to loss of hypoxic survival when LPS acute lung injury was combined with hypoxia (30).

Although hypoxia might be expected to activate HIF equally in all cell types present in the tissue, this is not always the case. Cell-type specific differences in HIF expression in lung tissue in response to alveolar hypoxia have been described (52). In a disease context, HIF-2 α is expressed in neutrophils infiltrating the airway of patients with COPD, but not in the epithelium (28). Alveolar macrophages in health exist at higher levels of ambient

Table 1. Hypoxia-inducible factor–hydroxylase pathway gene mutations and their consequences for innate immunity

Gene	Whole Organism Phenotype	Macrophage Phenotype	Neutrophil Phenotype
HIF-1	Hom: embryonic lethal Het: protected from SIRS response LysMcre: reduced inflammation, impaired host response to infection <i>ASO: reduced neutrophil recruitment, reduced inflammation, increased TB susceptibility</i>	Reduced glycolysis Reduced ATP stores Impaired bacterial killing Reduced cytokine levels	Reduced AMP release Reduced elastase Reduced NET formation No hypoxia survival response
HIF-2	Hom: embryonic lethal LysMcre: reduced inflammation, preserved host response Mouse gain of function: erythrocytosis and pulmonary hypertension <i>Whole animal gain of function: delayed inflammation resolution</i> Human gain of function: erythrocytosis, pulmonary hypertension	Reduced iNOS Reduced cytokine levels Reduced arginase	Increased sensitivity to nitrosative stress Gain of function delays spontaneous apoptosis
PHD1	Hom: reduced inflammation in colitis model	NK	NK
PHD2	Hom: embryonic lethal Het: reduced tumor metastasis	Increased M2 phenotype	NK
PHD3	Hom and LysMcre: reduced inflammation in hypoxia but increased SIRS response to sepsis	Increased HIF-1 expression Increased cytokine levels Increased phagocytosis	No hypoxic survival response
VHL	LysMcre: increased inflammation <i>Whole animal: erythrocytosis and increased ventilation</i> Human hets: no spontaneous phenotype but tumor syndromes with further somatic mutation in tissue	Increased HIF-1 expression Increased glycolysis	Het: delayed apoptosis in normoxia, increased phagocytosis
FIH	Hom: elevated metabolism and increased ventilation	NK	NK

Definition of abbreviations: AMP = anti-microbial peptide; ASO = morpholino antisense oligonucleotide; FIH = factor inhibiting HIF; het = heterozygote; HIF = hypoxia-inducible factor; hom = homozygote; iNOS = inducible nitric oxide synthase; LysMcre = myeloid-specific gene deletion; NET = neutrophil extracellular trap; NK = not known; PHD = prolyl hydroxylase; SIRS = systemic inflammatory response syndrome; TB = tuberculosis; VHL = von Hippel Lindau E3 ubiquitin ligase. Phenotypes are shown for murine (roman), zebrafish (italic), and human (bold) mutations in genes involved in the HIF hydroxylase signaling pathway. Unless otherwise specified, gene deletions are described.

oxygen than macrophages resident in other tissues, but how this affects the expression and activity levels of HIF pathway components has not been investigated, to our knowledge.

HIFs can also be highly expressed in tissue cells within the lung (e.g., in the submucosa of asthmatic airways [53]) and the airway epithelium of patients with chronic bronchitis (54). Expression of HIFs in tissue cells was shown to be protective in acute lung injury (55) and in the airway epithelium after oxidant stress (56), and pharmacologic studies are needed to determine whether or not the net effects of HIF antagonism in the context of inflammatory disease are beneficial.

Future Challenges

The emerging roles of the HIF-hydroxylase pathway in the regulation of inflammation are likely to be of particular relevance to

the lung, given the exposure of this tissue to very varied oxygen tensions in health and disease. Current understanding of the roles of different pathway components in the whole tissue is limited by use of cell-specific genetic modifications—despite their mechanistic usefulness in determining the contributions of different cell types to the overall *in vivo* phenotypes. Many studies combine mechanistic experiments in blood cells with whole animal models, but the behavior of an immune cell that has migrated into tissue may be very different. For example, hypoxia induces macrophage recruitment to the lung (48), and the phenotype of these newly migrated cells may well differ from that of macrophages differentiated *in vitro*. In the context of HIF-2 α deficiency, no neutrophil phenotype could be detected in blood cells, and accelerated neutrophil apoptosis was revealed only *in vivo* (28).

Although the feasibility of directly targeting HIF-1 may be reduced by its key

roles in host defense, through the identification of HIF-regulated genes (e.g., A2BAR [57]) it may be possible to identify downstream druggable targets. HIF-2 may itself be a potential target, given the dominance of its immunomodulatory role and its lesser effect on cellular energetics that are essential for host defense, although the antibacterial consequences of HIF-2 deficiency require further study. Specific targeting of HIF-2 using small molecules may be possible (58). There is also considerable interest in the potential of PHD inhibitors to enhance tissue protection by up-regulation of HIFs, reducing immune cell recruitment and thus ameliorating disease (59, 60). It will, however, be important to consider the potential proinflammatory consequences of HIF stabilization in immune cells. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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