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HIF-1 α : a master regulator of innate host defenses?

Kol A. Zarembler and Harry L. Malech

Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

In the days following infection, when the human body develops and refines antibodies and prepares to mount an adaptive immune response, the bulwark of innate host defense against microbial infection is the polymorphonuclear leukocyte (PMN). PMNs seek out, identify, engulf, and sterilize invading microbes using both O₂-dependent and O₂-independent antimicrobial systems. A decrease in PMN numbers or function caused by immunosuppression or disease increases the risk of infection. In this issue of the *JCI*, Peyssonnaud et al. identify a novel and essential role for hypoxia-inducible factor-1 α in regulating several important PMN functions relevant to host defense, including transcription of cationic antimicrobial polypeptides and induction of NO synthase (see the related article beginning on page 1806).

Hypoxia-inducible factor 1 (HIF-1) is a multisubunit protein that regulates transcription at hypoxia response elements (HREs) and is composed of 2 basic helix-loop-helix proteins: the α subunit, HIF-1 α , and the constitutively expressed HIF-1 β (also known as aryl hydrocarbon receptor nuclear translocator [ARNT]). As depicted in Figure 1A, during normoxia HIF-1 α is hydroxylated on several proline and asparaginyl residues, which enables high-affinity binding of HIF-1 α to von Hippel-Lindau tumor suppressor protein (vHL), a component of a ubiquitin ligase complex that ubiquitinates and thereby targets HIF-1 α for proteosomal degradation. Under hypoxic conditions the

O₂-dependent hydroxylation of HIF-1 α is decreased, which prevents its degradation (Figure 1B). A further level of O₂-dependent regulation exists: the hydroxylation of an asparagine residue by factor inhibiting HIF-1 α (FIH) blocks the interaction of HIF-1 α with p300/CBP transcriptional coactivator proteins, thereby decreasing transcription of HIF-1 α -regulated genes at normoxia. When HIF-1 α levels increase in response to hypoxia in tissues (or after stimulation of myeloid cells with bacteria, as discussed below), functional HIF-1 regulates transcription at HREs of target gene regulatory sequences, which results in the transcription of genes such as *Erythropoietin* and *VEGF* and thereby enhances local vascularization and systemic oxygen transport (Figure 1B). The details of the regulation of the general hypoxia response have been thoroughly discussed in several reviews (1–3).

A role for HIF-1 α in myeloid cells

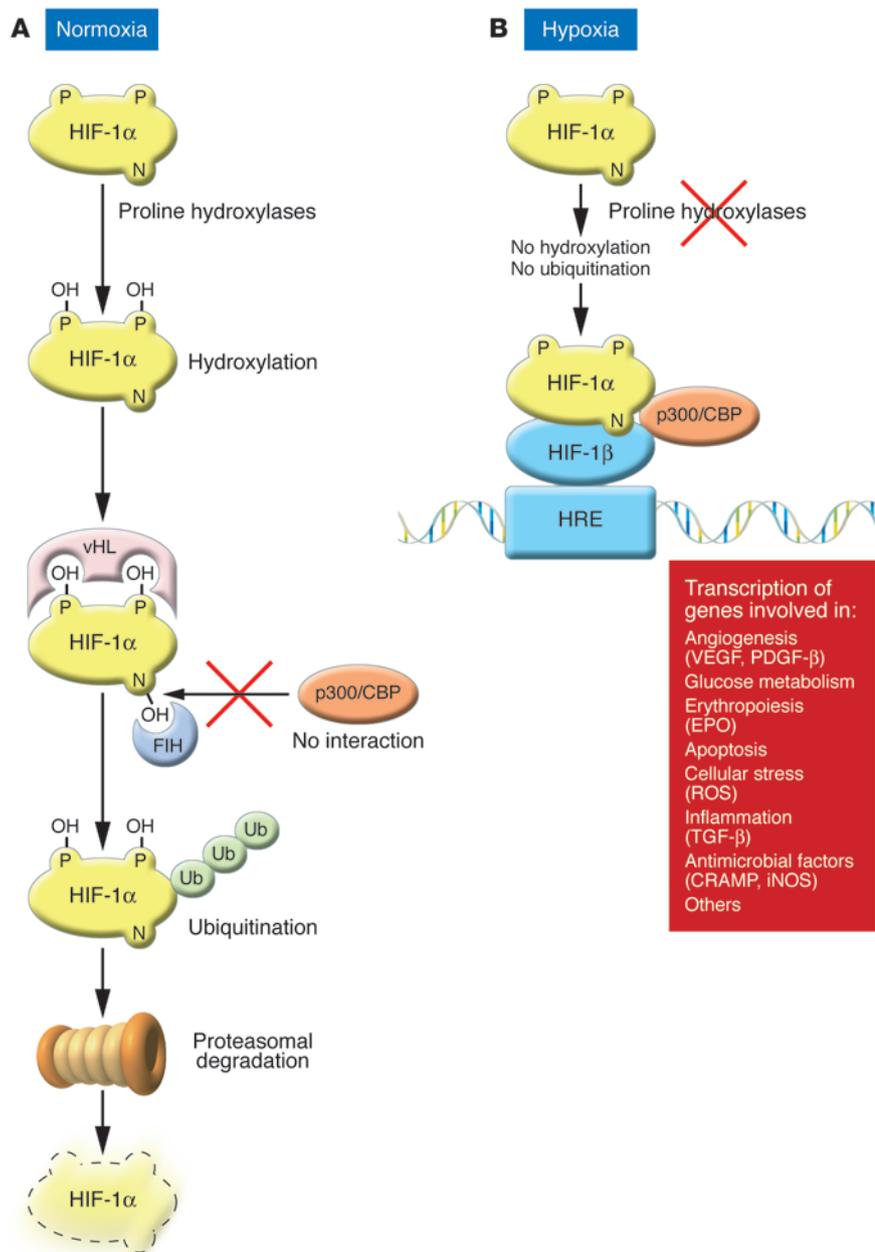
The knockout of HIF-1 α in mice is embryonically lethal due to its essential role in the development of the vascula-

ture. To circumvent this lethality and allow further examination of the role of HIF-1 α in phagocytes, Cramer et al. employed a myeloid cell-specific HIF-1 α -knockout approach and found that HIF-1 α was an important regulator of myeloid cell metabolism (by decreasing cellular ATP levels), neutrophil bactericidal potency, and macrophage migration (4). In this issue of the *JCI*, Peyssonnaud et al. (5) built on this foundation and attempted to understand the specific molecular defects contributing to depressed myeloid cell function in the absence of HIF-1 α . Temporarily leaving aside the consequences of the absence of HIF-1 α , the authors also discovered that in addition to being activated by hypoxia, HIF-1 α was equivalently upregulated in WT macrophages at normoxia following exposure to Gram-positive (*Streptococcus* and *Staphylococcus*) and Gram-negative (*Salmonella* and *Pseudomonas*) bacteria. HIF-1 α was previously shown to be activated in macrophages treated with LPS (6), a microbial activator of TLR-4. The 10 human TLRs are the cellular sentinels of microbial recognition. They respond to a variety of microbial products (e.g., LPS, lipoproteins, proteins, and nucleic acids) by activating signaling pathways leading to NF- κ B-mediated transcriptional regulation and, in some cases, activation of Rac1 and PI3K, which may regulate more rapid cellular responses (7). Regardless of the exact position along their signal cascades at which the TLR and HIF-1 α pathways intersect, these observations illuminate a fertile territory for further study. To what extent, if any, do the transcrip-

Nonstandard abbreviations used: CEBP, CCAAT enhancer-binding protein; CRAMP, cathelicidin-related antimicrobial peptide; FIH, factor inhibiting HIF-1 α ; HIF-1, hypoxia-inducible factor 1; HRE, hypoxic response element; PMN, polymorphonuclear leukocyte; vHL, von Hippel-Lindau tumor suppressor protein.

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**Figure 1**

HIF-1 α regulates several important PMN functions relevant to host defense during both normoxia and hypoxia. (A) During normoxia, O₂-dependent proline hydroxylases modify HIF-1 α proline residues 402 and 564. Asparagine 803 is hydroxylated by FIH, which decreases HIF-1 α interaction with the p300/CBP transcriptional coactivators. The hydroxylated prolines are recognized by vHL, a component of a ubiquitin ligase complex that ubiquitinates (Ub) HIF-1 α and thereby targets it for proteasomal degradation. (B) During hypoxia and/or bacterial infection, when proline hydroxylases are not active, HIF-1 α regulates transcription at HREs by accumulating and binding to HIF-1 β and p300/CBP, which results in transcription of hypoxia-inducible genes involved in angiogenesis, glucose transport and metabolism, erythropoiesis, inflammation, apoptosis, and cellular stress. EPO, erythropoietin.

tional programs regulated by HIF-1 and NF- κ B overlap and differ? Do all TLRs activate HIF-1 to the same extent?

In contrast to the findings of Cramer et al. (4), in which the recruitment of HIF-1 α ^{-/-}

polymorphonuclear lymphocytes (PMNs) to affected tissues was found to be defective in mice after exposure to a chemical irritant, Peyssonnaud et al. (5) found that HIF-1 α ^{-/-} PMNs were recruited to sites of

microbial infection *in vivo* as efficiently as were WT PMNs and were also equally able to generate ROS (a PMN microbicide). Interestingly, iNOS mRNA is induced by bacteria in a HIF-1 α -dependent fashion and nitrite production (a measure of NO production) is decreased in HIF-1 α ^{-/-} cells. NO itself acts as a microbicide, but the authors demonstrate that it was involved in regulating the expression of HIF-1 α . Further, both *in vivo* and *in vitro*, HIF-1 α ^{-/-} PMNs were less able to kill Gram-positive and -negative bacteria. This observation prompted Peyssonnaud et al. to examine the activity of one of the many known O₂-independent antimicrobial proteins, cathelicidin-related antimicrobial peptide (CRAMP). Cathelicidins were initially thought to be stored in PMNs as inactive proforms that required the actions of elastase, proteinase 3, or gastricsin to release a cationic C-terminal peptide with antimicrobial activity (8). However, recent studies have shown potentially important innate defense activities for unprocessed cathelicidins (e.g., LPS neutralization and chemoattractant activity). The results of Peyssonnaud et al. indicate that HIF-1 α is apparently required for the synthesis of CRAMP mRNA and protein, and deletion of vHL causes increased expression of CRAMP mRNA (Figure 1B). The cathelicidin-activating protease neutrophil elastase was found to be similarly regulated. Not only would reduced elastase activity decrease cathelicidin processing and antimicrobial activity (9), but elastase itself is directly antimicrobial (10). While HIF-1 α regulates cathelicidin expression, it has also been reported that a pig cathelicidin peptide, PR-39, can itself induce angiogenesis by inhibiting the degradation of HIF-1 α and subsequent induction of VEGF (11). The human cathelicidin-derived peptide LL-37 promotes angiogenesis in a VEGF-independent manner, and cathelicidin-deficient mice have impaired angiogenesis (12). If human LL-37 also upregulates HIF-1 α activity, its processing would promote further synthesis of cathelicidin, elastase, NO, and other HIF-1 α -regulated gene products as well as vascularization during wound repair.

In general, the pleiotropic effects of global regulators can complicate the dissociation of their primary (direct) effects from their secondary effects. No results of microarray analysis of gene expression profiles of HIF-1 α ^{-/-} myeloid cells have yet been reported; these may help identify



HIF-1 α -dependent transcription factors whose absence could be directly responsible for some of the observed defects in HIF-1 α KO PMNs. For example, it has been shown that mutations in the myeloid transcription factor CCAAT enhancer-binding protein- ϵ (CEBP- ϵ) can lead to secondary granule deficiency, a genetic disorder causing impaired bactericidal capacity in human PMNs and increased risk of infection (13), and are associated with decreased expression of antimicrobial proteins such as CRAMP (14).

vHL and immunity

Given the regulatory role of vHL in degrading HIF-1 α during normoxia, one would expect the absence of functional vHL to increase HIF-1 α levels and HIF-dependent transcripts. Peyssonnaud et al. (5) used vHL KO mice to confirm this prediction. It will be of interest to examine the PMNs of patients with von Hippel-Lindau disease in which the *VHL* gene is lost and test whether these cells are better able to kill microbes as suggested by the increased expression of cathelicidins and elastase observed in vHL-null neutrophils by Peyssonnaud et al. (5). Different mutations in vHL are associated with Chuvash polycythemia, a hypoxia-sensing disorder characterized by homozygous mutation of *VHL*. These patients exhibit increased erythropoietin and VEGF expression but they do not appear to suffer from increased tumorigenesis associated with von Hippel-Lindau disease (15), which suggests that additional signals emanate from vHL that may play important roles in its function as well as indirect roles in the function of HIF-1.

HIF-1 α and PMN longevity

The PMN is a relatively short-lived cell (its half-life in the circulation is about 6 hours) and it exits the bone marrow replete with antimicrobial factors stored in cytoplasmic granules. The few ribosomes and scant ER possessed by mature PMNs has led to the general impression that the biosynthetic capacity of the PMN is limited. However, the PMN's lifespan is increased significantly by inflammatory stimuli such as LPS and IL-1 (16). In addition, it was recently shown that hypoxia actually delays apoptosis of PMNs in a HIF-1 α -dependent fashion (17, 18), so that the same signals that induce the expression of CRAMP mRNA

— and other HIF-1 α -dependent proteins expressed by PMNs — would also allow the PMN to survive (6) and perhaps allow sufficient time for the limited biosynthetic capacity of these cells to contribute functionally to innate immune defense.

PMNs accumulate at sites of inflammation or infection and when activated they release antimicrobial factors, including ROS such as H₂O₂ and HOCl (the active ingredient in household bleach), which promote the killing of extracellular microbes. ROS are generated by NADPH oxidase systems in PMNs in a process referred to as the respiratory burst. Peyssonnaud et al. (5) found that HIF-1 α ^{-/-} PMNs generated normal levels of ROS. It has been previously recognized, and is also shown in the current study, that inflammatory loci become hypoxic in vivo. Indeed the respiratory burst of PMNs was first recognized by a decrease in O₂ levels measured using a Warburg manometer (19). It may well be that HIF-1 α is activated in vivo by this depression in local O₂ (in addition to bacteria- and TLR agonist-induced activation), which leads to increased synthesis of O₂-independent antimicrobial polypeptides (such as cathelicidins) by PMNs. It will be very interesting to see if patients with chronic granulomatous disease, whose myeloid cells lack the enzymatic machinery required to generate a respiratory burst, are consequently unable to generate a second signal that would normally lead to HIF-1 α -mediated induction of O₂-independent antimicrobial systems.

By dissecting the role of HIF-1 α in innate immune defenses, the study by Peyssonnaud et al. (5) introduces new targets for therapeutic immunomodulation. Several compounds found to activate HIF-1 α in vitro have been used in the clinic for other purposes and appear to be well tolerated (e.g., mimosine, CoCl₂, and desferrioxamine). These compounds may increase the production of cationic antimicrobial polypeptides and NO through activation of HIF-1 α and thereby augment the production of endogenous antibiotics. The complex web of associations of HIF-1 α with the TLR signaling pathway and with vHL may provide abundant opportunities for therapeutic intervention in immune and inflammatory disorders, cancer, and vascular development. Further studies of the ways in which HIF-1 α

orchestrates effective innate immune defenses are therefore urgently needed.

Address correspondence to: Harry L. Malech, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive, MSC 1456, Bethesda, Maryland 20892-1456, USA. Phone: (301) 402-1802; Fax: (301) 402-0789; E-mail: HMALECH@niaid.nih.gov.

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