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REVIEW

Hypoxia inducible factor (HIF) function in innate immunity and infection

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Abstract The hypoxia-inducible transcription factor (HIF-1α) is a major regulator of energy homeostasis and cellular adaptation to low oxygen stress. Recently, HIF-1α has been discovered to function as a global regulator of macrophage and neutrophil inflammatory and innate immune functions, as befits these specialized phagocytic cells who must operate effectively in the hypoxic microenvironments of infected tissues. This review summarizes current knowledge of the role of HIF-1α in mammalian innate immunity, emphasizing insight gained from conditional gene targeting of the transcription factor in the myeloid cell lineage. Dynamic changes in HIF-1α expression in the course of bacterial, viral, or parasitic infections are outlined and inferences drawn regarding the consequences for pathogen and host. A better understanding of HIF-1α function may provide novel and rational approaches for boosting innate immune function in the therapy of certain complicated infectious disease conditions.

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Introduction

Microorganisms are omnipresent, colonizing the exterior and interior epithelial surfaces of the human host. This constant interaction leads to many host benefits, for example vitamin production, but also significant disadvantages, such as the risk of infection. To control this fragile balance, the host has evolved a broad arsenal of innate defense mechanisms, including physical barrier functions, soluble effectors such as complement and antimicrobial peptides, and phagocytic cells. The critical role of phagocytes in host defense lies in their rapid mobilization and ability to recognize and inactivate pathogens independent of prior encounter as required by adaptive immunity.

The principal phagocytes of mammalian innate immunity are cells of the myeloid lineage, monocyte/macrophages and neutrophils. These short-lived cells are recruited in response to alterations in tissue integrity, whether the byproduct of chemical or physical injury or the spread of infectious microorganisms. In concert with vasodilation and increased vascular permeability, the activated phagocytes contribute to local inflammation. Tissue foci of inflammation are characterized by low levels of oxygen and glucose, together with high concentrations of lactate and reductive metabolites, including free oxygen radicals [1, 2]. To maintain energy homeostasis and carry out their biological activities in these specialized environments, phagocytic cell types must generate ATP via glycolysis. Because upregulation of virtually every enzyme in the glycolytic pathway is mediated almost exclusively by the hypoxia-inducible factor-1α (HIF-1α) [3], a role for this transcription factor in

supporting phagocyte function during inflammation was intuitive. In the last few years, genetic tools provided not only experimental validation of this concept, but uncovered profound implications of the HIF-1α control pathway in the overall regulation of mammalian innate immunity.

HIF-1α and myeloid cell inflammation

HIF-1 is a heterodimeric helix-loop-helix transcription factor whose expression is regulated by oxygen at the protein level. Protein stability of the α subunit (HIF-1α) is regulated by a family of oxygen- and iron-dependent prolyl hydroxylases, whose action directs HIF-1α for degradation by the ubiquitin–proteasome pathway in a process dependent upon interaction with von Hippel–Lindau tumor-suppressor protein (vHL). Under hypoxia, prolyl hydroxylase activity is inhibited, HIF-1α accumulates and translocates into the nucleus, where it binds the constitutively expressed HIF-1β (aka ARNT). The resultant heterodimer HIF-1 binds to hypoxic response elements (HREs) of target gene regulatory sequences. Classical HIF-1 target genes include the glycolytic enzymes, glucose transporters, erythropoietin, and the angiogenic factor VEGF.

Gene targeting provides invaluable evidence about the function of specific mammalian proteins, but global elimination of HIF-1α in the mouse produced lethal embryonic defects in vascularization and morphologic development [4–6]. The advent of conditional gene targeting methodologies allowed tissue-specific deletion of floxed HIF-1α or vHL alleles in the myeloid lineage (macrophages and neutrophils) by crosses into a background of cre recombinase expression driven by the lysozyme M (lysM) promoter. The resultant mice, with either deficient or exaggerated HIF-1α expression in their phagocytes, are phenotypically normal at baseline but display marked abnormalities when examined in classical models of inflammation [7]. Compared to wild-type mice, HIF-1α myeloid-null animals have less skin redness and edema after irritation with detergent solution, and greatly diminished joint swelling and cartilage destruction in a passive serum-induced arthritis model [7]. A role for HIF-1α in chronic inflammatory diseases such as rheumatoid arthritis and atherosclerosis is further corroborated by elevated levels of the transcription factor in biopsies of primary lesions of patients [8–11].

HIF-1α: a master regulator of innate immunity

The contribution of HIF-1α to myeloid cell-mediated inflammatory pathologies prompted immediate consideration of the capability of the transcription factor to mediate central functions of macrophages and neutrophils in innate host defense. Whereas in healthy tissues, oxygen tension is 17.5–63 mm Hg (i.e., 2.5–9% oxygen), much lower levels (<1% oxygen) are present in wounds and tissue foci of infection. Many bacterial pathogens survive well and proliferate under anaerobic conditions, thus phagocytes must be adapted to function effectively in microbial eradication in the same microenvironments. Genetic experiments in which mouse phagocyte HIF-1α levels were manipulated revealed the pivotal role of the transcriptional control pathway in phagocyte bactericidal activity.

Macrophages from mice deficient in HIF-1α show diminished capacity to kill Gram-negative and Gram-positive bacteria when compared to macrophages from wild-type littermates [7, 12], and HIF-1α deficient animals were more susceptible to invasive skin infection produced by *Streptococcus pyogenes*. While HIF-1α deletion (or overexpression through vHL elimination) did not alter phagocyte production of reactive oxygen species through the respiratory burst, the expression of a number of other molecular effectors of host innate defense was significantly correlated to HIF-1α levels. These included cathelicidin antimicrobial peptides, the granule proteases cathepsin G and elastase, tumor necrosis factor-α (TNFα), and nitric oxide (NO) produced by the inducible NO synthetase (iNOS) [12]. In addition to its microbicidal properties, NO is known to stabilize HIF-1α by redistributing intracellular oxygen and inhibit prolyl hydroxylase activity [13], thereby setting up an autocrine feedback loop to amplify myeloid cell inflammatory activation.

A paradoxical result of these findings is that, due to HIF-1α activation, macrophages actually phagocytose and kill bacteria better under hypoxic conditions than they do under normoxic conditions [12, 14]. Even more striking, bacterial exposure is a stronger stimulus for HIF-1α stabilization than is hypoxia itself, and bacterial-induced HIF-1α stabilization is readily demonstrated even at normoxia [12]. HIF-1 induces leukocyte β2 integrin expression and thereby can promote neutrophils binding to epithelium [15], and inhibits neutrophil apoptosis via NF-κB activity [16, 17], thereby prolonging the effective window for these cells to participate in phagocytic killing. Upregulation of HIF-1α has also been demonstrated during the differentiation of monocytes circulating in the blood to tissue bound macrophages [18].

Because circulating phagocytes serve a unique biological role, and must pass through radically different microenvironments upon their rapid mobilization to infected tissues, the HIF-1α pathway represents an elegant control mechanism for the specialized functions of these cells (Fig. 1). Phagocyte bactericidal and proinflammatory capacities can be maintained in an "off" state while the myeloid cells circulate in the oxygen-rich bloodstream; and then be rapidly activated in response to the declining oxygen gradient encountered upon diapedesis and entry of

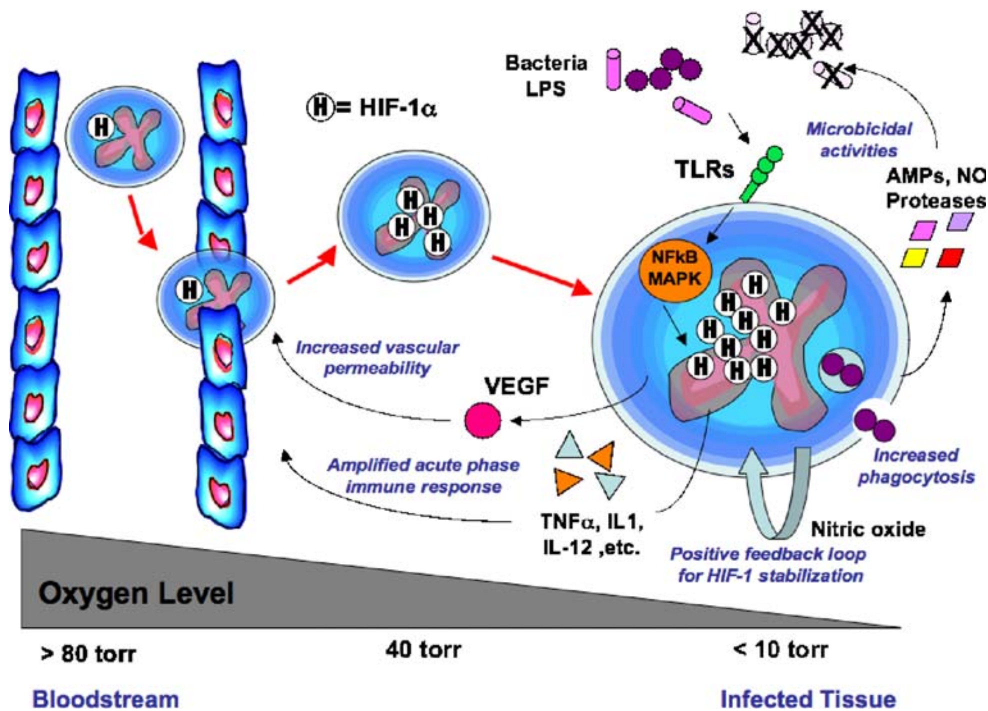

Fig. 1 Model for HIF-1α transcriptional regulation of phagocyte innate immune functions. Phagocytic cells such as neutrophils and macrophages have low HIF-1α levels when circulating in the oxygen-rich bloodstream. When recruited to tissue foci of inflammation, they encounter a declining oxygen gradient which increases cellular HIF-1α levels and initiates activation of proinflammatory and bactericidal effector genes bearing HREs. Maximum activation is achieved on bacterial encounter, where HIF-1α expression is further stimulated through pattern recognition receptors (e.g., TLR-4) and cell signaling pathways (e.g., NFκB, MAPK) just beginning to be elucidated. HIF-1α promotes increased phagocytosis and the release of antimicrobial peptides (e.g., cathelidicins) and granule proteases with direct microbicidal activities. Increased VEGF production and elaboration of proinflammatory cytokines facilitate recruitment and activation of additional immune effector cells. Activation of inducible nitric oxide (NO) synthetase generates a molecule (NO) with not only direct antimicrobial properties, but also the ability to further stabilize HIF-1α and rapidly amplify the innate defense pathway in the phagocyte (modified and updated from [11])

the cells into the infected tissues. The primed phagocyte then encounters a further potent stimulation of the HIF-1α transcriptonal pathway by direct encounter with the bacteria, as potentiated by the NO-mediated amplification loop. This regulatory mechanism underlying HIF-1α control of target genes involved in microbial killing ensures that the corresponding inflammatory mediators are expressed preferentially in tissue foci of infection but not in healthy tissues where inflammatory damage might otherwise harm host cells.

New evidence indicates that HIF-1α control of genes with innate immune functions may not be limited to phagocytic cell types, as conditional gene targeting and RNAi inhibition of HIF-1α in skin keratinocytes decreased their production of antimicrobial peptides and intrinsic capability to control bacterial proliferation [19]. HIF-1α transcriptional regulation also appears to modulate the production of proinflammatory cytokines in CD4+ and CD8+ T lymphocytes, but in contrast to findings in macrophage and neutrophil lineages, the effect may be suppressive. After T-cell receptor activation, the release of TNFα and interferon γ (IFNγ) was increased in T cells with targeted deletion of the HIF-1α gene compared to wild-type T cells [20]. The release of T cells from HIF-1α suppression enhances their proliferation and allows them to contribute proinflammatory cytokine production and restrict bacterial proliferation in a cecal ligation puncture model [21].

Finally, it has recently been discovered that HIF-1α transcriptional regulation mediates allele expression phenotypes for the innate defense factor SLC11A1 (aka NRAMP1) through differential binding and activation of Z-DNA forming microsatellite polymorphisms in the SLC11A1 promotor [22]. Since these polymorphisms influence human susceptibility to tuberculosis, rheumatoid arthritis, Crohn's disease and other disorders, HIF-1α may influence heritable variation in innate resistance to infection and inflammation within and between populations [22].

HIF-1α activities during bacterial infection

Explorations of HIF-1α function in activating neutrophil and macrophage bactericidal activities demonstrated increased levels of the transcription factor were stimulated in response

to a variety of bacterial species including *Streptococcus pyogenes*, *Streptococcus agalactiae, Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* [7, 12, 22, 23], suggesting it subserves a general role in bacterial/host interactions. Loss of myeloid cell HIF-1α renders mice more susceptible to invasive *S. pyogenes* infection [12], and decreases phagocyte killing of Gram-negative and Gram-positive bacteria in vitro [7, 12, 23], suggesting that the HIF-1α response pathway is broadly adaptive in host defense. In select additional research studies, the interplay between a specific bacterial infection and HIF-1α expression has been pursued in more detail.

One clear demonstration of HIF-1α induction by a bacterial pathogen was provided in studies of *Bartonella henselae*, a facultative intracellular bacterium that causes cat-scratch disease and, in immunocompromised patients, the angioproliferative disorder known as bacillary angiomatosis (BA). Using HeLa as a model host cell line, *B. henselae* infection was found to induce HIF-1α by Western blot and electrophoretic mobility shift assays, and immunoflourescence studies of BA lesions from human patients showed high levels of HIF-1α expression [24]. Mutant studies suggest that pilus appendages on the *B. henselae* surface contribute to HIF-1α activation, VEGF secretion and initiation of a proangiogenic program that characterizes BA tissue pathology [24]. Another intracellular pathogen, *Chlamydia pneumoniae*, appears to have evolved a unique mechanism to counteract HIF-1α stabilization, thereby blunting innate immune function and promoting its own survival within host cells. During the later phase of intracellular chlamydial replication (48–72 h), secretion of the chlamydial protease-like activity factor into the host cell cytoplasm degraded accumulated HIF-1α, and this proteolytic activity was shown to play an essential role in *C. pneumoniae* replication during hypoxia [25].

Finally, the opportunistic pathogen *P. aeruginosa* has evolved mechanisms to sense alterations in host immune function and activation, and respond in a fashion that increases its virulence phenotypes, including expression of the PA-1 lectin/adhesin, a protein capable of inducing defects in host epithelial barrier function [26]. HIF-1α is upregulated in intestinal epithelial cells exposed to hypoxia, and stimulates the release of adenosine, which normally exerts a cytoprotective function. *P. aeruginosa* is able to metabolize adenosine to inosine via adenosine deaminase, and both adenosine and inosine upregulate PA-1 lectin/adhesin expression by the bacterium in a dose-dependent fashion [27]. In this sense, the pathogen senses HIF-1α dependent changes in host cell function and subverts innate defense by virulence factor upregulation and metabolic elimination of a cytoprotective factor.

Since the mechanism of HIF-1α turnover involves iron-dependent prolyl hydroxylase activity, it is conceivable that bacterial sequestration of iron could have the effect of HIF-1α stabilization. Preliminary data have emerged that this phenomenon may operate in the Peyer's patches of the intestine. Pathogens including *Yersinia enterocolitica*, *Salmonella enterica* or *Enterobacter aerogenes* induce HIF-1α expression in Peyer's patches, and this induction can be replicated by application of purified siderophores (iron-binding proteins) from each species [28]. In contrast, siderophore-deficient bacterial mutants fail to induce HIF-1α activation. The disease potential of *Y. enterocolitica* is enhanced in mice deficient in intestinal HIF-1α expression, suggesting this activation pathway contributes to host innate defense [28].

HIF-1α dynamics in viral infection

Activation of HIF-1α pathway during the life cycle of viral pathogens has been the subject of increasing investigation, revealing a diversity of functional outcomes in disease progression and critical linkages to viral oncogenesis. Viral infection is generally appreciated to induce stabilization of HIF-1α in target cells, which consequently contributes to local inflammation. For example, the common upper respiratory tract pathogen respiratory syncytial virus (RSV) induces HIF-1α in primary human bronchial epithelial cells via a NO-dependent pathway [29]. Increased HIF-1α levels stimulates VEGF production, enhancing monolayer permeability, which may play a role in the airway edema of acute RSV infection.

In some cases, HIF-1α may help coordinate a host defense program to limit cell damage secondary to viral infection. This scenario might particularly apply to viruses which exert an acute cytolytic effect, such as the vesicular stomatitis virus (VSV). Several years ago, it was noticed that hypoxia (2% O_2, 14 mmHg) reduced the cytopathogenicity and replication of VSV, with measured antiviral effects of interferons α and γ potentiated under the low oxygen conditions [30]. Interferons in turn have been shown to upregulate the expression of HIF-1α [31]. The significance of HIF in the antiviral response to VSV was recently established by a pharmacological approach – inhibition of HIF activity by a small molecule antagonist (chemotin) or RNA interference enhanced VSV cytotoxicity and replication, whereas the treatment with the hypoxia mimetic cobalt chloride promoted cellular resistance to infection [32]. Furthermore, expression profiling showed HIF enhancement of interferon β and other antiviral genes during VSV infection [32].

For a number of persistent viral infections, when induction of HIF-1α is insufficient to effect eradication, the accompanying proangiogenic program can contribute to oncogenesis. For example, chronic infections with the hepatitis B and C viruses (HBV and HCV) are epidemio-

logically associated with development of hepatocellular carcinoma (HCC), a highly vascularized solid tumor. The X protein of HBV (HBx) is felt to play an important role in angiogenesis and metastasis of HCC [33]. HIF-1α levels and nuclear translocation are increased in cultured liver cells by expression of HBx via p42/p44 mitogen-activated protein kinase (MAPK) pathways, leading to transcriptional activation of HIF-1α target genes including VEGF, a finding corroborated in vivo in the livers of HBx transgenic mice [34]. Immunohistochemical studies in these mice reveal increased microvessels in dysplastic areas of liver where HIF-1α, VEGF, and HBx co-localize [35]. HBx was found to interact directly with the bHLH/PAS domain of HIF-1α, blocking its association with vHL and thus preventing its ubiquitin-mediated degradation [35]. Moreover, HIF-1α activation induced by HBx was found to increase the multi-drug resistance 1 (MDR1) transporter activity in a hepatoma cell line, thereby contributing to increased resistance of the cancer cells to chemotherapeutic agents [36]. Recently, HCV infection has also been found to stabilize HIF-1α with contributions from MAPK and other cellular activation pathways (NF-kB, STAT-3, PI3-K/AkT), stimulating VEGF production and neovascularization in a chick chorioallantioic membrane surrogate assay system [37].

Another example linking viral activation of HIF-1α to proangiongenesis and tumor development can be found in the case of human papillomavirus-16 (HPV-16), an etiologic agent of cervical interstitial neoplasia that, undetected, can progress to cervical carcinoma. In advanced cervical cancer lesions, many of which are hypoxic, increased HIF-1α levels can be correlated to poor prognosis [38, 39]. Transgenic mice expressing HPV-16 in cervical epithelium under control of the K14 promotor can develop locally invasive cervical cancers; the size of these lesions is increased 70-fold when double transgenic mice are created with both HPV-16 and HIF-1α expression driven by K14 [40]. Transfection of human cervical cancer cell lines with HPV-16 oncoproteins E6 and E7 can induce VEGF expression and capillary formation in vitro; however, this proangiogenic effect is abolished when the cells are co-transfected with siRNA targeting HIF-1α [41]. Together, these new studies suggest a synergism of HIF-1α with viral ongogenes in premalignant lesions to promote gene activation programs favoring neovascularization and cancer development.

HIF-1α activation is apparent during other viral infections associated with risk of neoplastic transformation. The retrovirus human T-cell leukemia virus type 1 (HTLV-1) causes adult T-cell leukemia. Increased HIF-1α protein levels and VEGF expression are detected in T-cell lines infected with HTLV-1, via a process involving activation of PI3K/Akt signaling by the HTLV-1 protein Tax [42]. The herpesvirus Epstein Barr virus (EBV) is linked to development of nasopharyngeal carcinoma in specific populations. The major oncoprotein of EBV, latent membrane protein 1 (LMP1), increases HIF-1α levels and stimulates VEGF expression in nasopharyngeal epithelial cells through a mechanism dependant both upon H_2O_2 production and p42/p44 MAPK activity [43]. Recent co-immunoprecipation studies indicate EBV LMP1 enhances the stability of Siah1 E3 ubiquitin ligase, inducing proteasomal degradation of PHD-1 and PHD-3 that normally tag HIF-1α for degradation. LMP1 thus prevents formation of the VHL/HIF-1α complex, providing a mechanism for HIF-1α stabilization during EBV nasopharyngeal cell infection [44].

A fascinating crosstalk between viral genes and the HIF-1α pathway has recently been elucidated for the human herpesvirus 8 (HHV-8). Latent infection with HHV-8 is associated with the endothelial tumor Kaposi's sarcoma (KS) in AIDS patients and others with lowered immunity, and KS lesions in vivo are associated with high levels of both the HIF-1α and HIF-2α protein [45]. HHV-8 infection of endothelial cells in vitro leads to increased stabilization of the two HIF α subunits and increased HIF-responsive gene expression [45]. The HHV-8 latency-associated nuclear antigen (LANA), which plays a critical role in modulating viral and target cell gene expression, increases HIF-1α mRNA levels, and also physically interacts with the transcription factor to enhance its promoter activities [46]. HIF-1α induction and co-activation during HHV-8 infection can then lead to activation of target genes in the genome of the virus itself, including that encoding Rta, which is involved in transition of the virus from latency to a lytic replication phase. Several putative HREs are identified in the essential Rta promoter, and HIF-1α-dependent binding of a LANA protein complex to such HREs can be demonstrated by electromobility shift assays [46]. In another potential example of viral-HIF crosstalk, replication of parvovirus B19, a cause of erythema infectiousum or “fifth disease”, is enhanced under hypoxic conditions. HIF-1α binding to an HRE located in the B19 promoter region can be demonstrated; however, the full implications of this finding for viral pathogenesis remain unclear [47].

HIF-1α modulation in parasitic infection

Though much less well studied, induction of HIF-1α also appears to occur in the context of infection with protozoan parasites. For example, cutaneous lesions can be generated in BALB/c mice by infection with *Leishmania amazonensis*, and in the later stages of infection, HIF-1α induction in the cytoplasm and parasitophorous vacuoles of macrophages recruited to the microenvironment can be clearly demonstrated [48]. An interesting comparative microarray analysis of transcriptional activation patterns in human fibroblasts infected with the obligate intracellular parasites *Toxoplasma*

gondii and *Trypanosoma cruzi* has identified an important role for HIF-1α in the life cycle of the former. *T. gondii*, a cause of opportunistic infections in fetuses and the immunocompromised, induced transcripts for glycolytic enzymes, glucose transporters, transferrin receptor and VEGF, a transcriptome signature for HIF-1α activation [49, 50], whereas parallel fibroblast infection studies with *T. cruzi* produced minimal changes in expression profile [51]. Further investigation showed *T. gondii* infection rapidly induced levels of HIF-1α and activated reporter gene expression in infected fibroblasts [52]. Under hypoxic conditions (3% oxygen) present in the tissues (brain, muscle, retina) in which the parasite produces serious disease, *T. gondii* cell division and organelle maintenance was severely impaired in host cells where HIF-1α function was deleted. The *T. gondii* parasite may have evolved to induce HIF-1α because a target gene is essential to parasite growth, or alternatively because HIF-1α activation is necessary to preserve the health of the host cell in which the parasite has become established [52].

HIF-1α and sepsis

Sepsis reflects a maladaptive and potentially lethal host response to infection, in which bacteria or lipopolysaccharide (LPS) act as potent activators of uncontrolled proinflammatory cytokine release from immune cells including monocytes and macrophages. LPS raises levels of HIF-1α in macrophages through activities of the p42/44 MAPK and NfkB signal transduction pathways [53] and in hepatocytes by JNK signaling and c-Jun activation [54]. Recently, it has been shown that LPS increases HIF-1α and decreases prolyl hydroxylase mRNA production through a process that depends upon LPS pattern recognition by Toll-like receptor 4 (TLR-4) [55]. Studies of LPS challenge in mice with conditional gene targeting of HIF-1α in the myeloid lineage reveal HIF-1α to be a critical determinant of the sepsis phenotype, promoting high level production of inflammatory cytokines TNF-α, interleukin-1 (IL-1), IL-6, and IL-12. HIF-1α deletion in the macrophage lineage is protective against LPS-induced mortality and blocks development of clinical indicators of sepsis including hypothermia, tachycardia, and hypotension [55].

HIF-1α: a therapeutic target for infectious diseases?

We have summarized above an evolving view of HIF-1α as a master regulator of the innate immune function of phagocytes [12, 56, 57]. Since pharmacological approaches for manipulating HIF-1α levels have been considered extensively in the context of cancer therapy and angiogenesis [58, 59], the possibility is raised of a novel approach to therapy of infectious disease conditions, namely boosting the bactericidal capacity of phagocytes [60]. Conceptual support for this concept was provided by in vitro studies where genetic augmentation of macrophage HIF-1α levels (through vHL deletion) or addition of a series of pharmacological agonists of HIF-1α (hypoxia mimetics that restrict prolyl hydroxylase access to iron), each enhanced murine macrophage bactericidal activity [12]. Similarly, dose-dependent enhancement of the bactericidal activity of human whole blood, neutrophils, and macrophage cell line U937 against the pathogen *Staphylcococcus aureus* was achieved using the HIF-1α agonist L-mimosine [23]. Local treatment with L-mimosine also significantly delayed the progression of *S. aureus* skin abscesses in a murine challenge model [23].

The proof-of-principle experiments described suggest further exploration of HIF-1α augmentation to boost innate defense function. This may be of interest as a therapeutic strategy in infectious disease conditions complicated by antibiotic resistance or compromised host immunity (e.g. AIDS, cancer chemotherapy). An advantage of targeting host molecules that enhance phagocyte recruitment or activate array of phagocyte bactericidal mechanisms is lack of selective pressure for resistance – the bacterial pathogen cannot evolve to combat a drug that targets the host and effectively deploys a multifaceted combination therapy of natural antimicrobial molecules [61]. Coincidently, HIF-1α agonist therapy may benefit the anemia that can accompany chronic infection, since it functions in the liver to suppress production of the peptide hepcidin, promoting iron absorption and mobilization, and stimulates red blood cell synthesis by increasing EPO production [62]. Finally, strategies to inhibit HIF-1α in therapy of chronic inflammatory disorder such as rheumatoid arthritis may provide a safer therapeutic margin than high-dose steroids or cytotoxic agents, since rapid posttranslational regulation of HIF-1α levels could allow rapid restoration of innate immune function of phagocytes upon drug withdrawal in the event of opportunistic infection.

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Conflict of interest statement The authors declare no conflict of interest.

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