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## Interdependence of hypoxic and innate immune responses

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### Abstract

Hypoxia-inducible transcription factor (HIF) is a major regulator of cellular metabolism and adaptation to cellular stress caused by oxygen deficiency (hypoxia). Phagocytic cells have an essential role in innate immune defense against pathogens; and this is a battle that takes place mainly in the hypoxic microenvironments of infected tissues. It has now become clear that HIF promotes the bactericidal activities of phagocytic cells and supports the innate immune functions of dendritic cells, mast cells and epithelial cells. In response to microbial pathogens, HIF expression is upregulated through pathways involving the key immune response regulator nuclear factor- $\kappa$ B, highlighting an interdependence of the innate immune and hypoxic responses to infection and tissue damage. In turn, HIF-driven innate immune responses have important consequences for both the pathogen and the host, and its central role in immune defence demonstrates the importance of the tissue microenvironment in controlling infectious disease.

### Keywords

HIF-1; Hypoxia; Innate Immunity; Phagocyte; Neutrophil; Macrophage; NF- $\kappa$ B

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Acute tissue foci of inflammation, whether generated in response to infection, injury, noxious agents or autoimmunity, present a unique and challenging microenvironment. Hypoxia (low oxygen) or anoxia (complete lack of oxygen), hypoglycaemia (low blood glucose), acidosis (high H<sup>+</sup> concentration) and abundant free oxygen radicals are characteristic features of inflamed tissues, along with the influx of specialized myeloid cells such as neutrophils and macrophages<sup>1,2</sup>. These short-lived cells are rapidly mobilized in response to any change in tissue integrity or entry of pathogenic microorganisms. They carry out phagocytosis and release a diverse array of antimicrobial molecules and pro-inflammatory mediators. Neutrophils and macrophages are crucial elements of innate immune defense, serving to localize and eradicate pathogens and prevent the systemic spread of infection. Quantitative deficiencies in the numbers of these specialized phagocytic

cell types (for example, following cancer chemotherapy) or inherited defects in their core effector functions (for example, in chronic granulomatous disease) greatly increase susceptibility to recurrent or severe infections.

The intricate regulation of the microbicidal and inflammatory functions of neutrophils and macrophages is central to our understanding of mammalian innate immunity. Innate immune cells must remain quiescent at baseline to avoid unwanted inflammatory injury to normal host tissues, yet be capable of instantaneous activation when recruited to sites of infection. Extensive investigations have defined cell surface receptors and downstream signalling pathways that allow these cells to rapidly activate gene transcription and to release pre-formed antimicrobial effectors following recognition of pathogens or cytokines. Improved understanding is emerging of how another cardinal feature of the inflammatory tissue microenvironment, the scarcity of oxygen, influences the terms of engagement between phagocytic cells and pathogens. In this Review, we describe how these frontline innate immune effector cells have evolved to generate energy and carry out their microbicidal functions under hypoxic conditions. From this fundamental need emerges an interdependence of the innate immune response and the hypoxic response, revealing a central role of the hypoxia-inducible transcription factors (HIF) as regulators of mammalian innate defense.

## HIF: A central regulator of the hypoxic response

The HIF protein complex was originally discovered for its contribution to the dramatic induction of erythropoietin (EPO) gene transcription under hypoxic conditions<sup>3, 4</sup>. Further characterization revealed HIF as a heterodimeric helix-loop-helix transcription factor, the expression of which is regulated by both oxygen and iron (Figure 1). HIF is active in all mammalian cells, and is now known to regulate the expression of more than 100 genes that function in a wide variety of host cellular and systemic responses to stress triggered by low oxygen levels. HIF-controlled pathways influence metabolism, angiogenesis, vascular tone, cell differentiation and apoptosis with implications for normal physiology and development but also for cancer and many other pathological conditions<sup>5, 6</sup>.

The HIF DNA-binding complex is comprised of the constitutively expressed HIF1 $\beta$  subunit that partners with one of two hypoxia-inducible  $\alpha$ -subunits, HIF1 $\alpha$  or HIF2 $\alpha$ <sup>4</sup>. The HIF  $\alpha$ -subunits are unstable under normoxic conditions; cells continually synthesize and degrade these proteins. The short half-lives of HIF1 $\alpha$  or HIF2 $\alpha$  are the by-product of the activities of a family of oxygen- and iron-dependent prolyl hydroxylases (PHD1, PHD2 and PHD3)<sup>7</sup>, the actions of which direct HIF  $\alpha$ -subunits for degradation by the ubiquitin–proteasome pathway [G] in a process that depends on interaction with von Hippel–Lindau tumour-suppressor protein (VHL)<sup>8</sup>. However, under conditions of hypoxia or iron deprivation, prolyl hydroxylase activity is inhibited, and HIF1 $\alpha$  or HIF2 $\alpha$  can accumulate and translocate to the nucleus, where they bind HIF1 $\beta$  (Figure 1). The resultant heterodimeric HIF complex binds to core pentanucleotide sequences known as hypoxic response elements (HREs) in the promoter regions of target genes. Classical HIF target genes include most of the glycolytic enzymes, glucose transporters, erythropoietin, and the angiogenic factor vascular endothelial growth factor (VEGF). The HIF-regulated genes function in concert to decrease

mitochondrial oxygen consumption<sup>9</sup>, orchestrate the metabolic shift to anaerobic glycolysis<sup>10</sup> and balance cellular pH owing to increased lactic acid production<sup>11</sup> – thus optimizing cell energetics and homeostasis for survival and function in oxygen-poor environments.

## HIF function in myeloid cells

### HIF control of myeloid cell inflammatory activities

Given its central roles in development and physiology, global deletion of HIF1 $\alpha$  in the mouse resulted in lethal embryonic defects in vascular development and morphology<sup>12–14</sup>. Recently, application of a conditional gene targeting strategy exploiting Cre-Lox recombination succeeded in lineage-specific elimination of HIF1 $\alpha$  in macrophages and neutrophils. A mouse line was engineered to possess *loxP* sites flanking the *Hif1a* gene, and these mice were crossed into a mouse background where the myeloid-cell specific lysozyme M (lysM) promoter drives Cre recombinase expression. The resultant mice, with markedly reduced HIF1 $\alpha$  expression levels specific to their myeloid cells, have no obvious phenotypic abnormalities at baseline, but showed marked aberrations in experimental models of acute inflammation<sup>15</sup>. In contrast to their wild-type littermates, myeloid cell *Hif1a* conditional knockout mice did not develop severe joint swelling and cartilage destruction in a collagen-induced arthritis model, and showed no cutaneous redness or oedema following application of a chemical irritant to the skin, suggesting impaired inflammatory responses<sup>15</sup>. The HIF1 $\alpha$ -deficient macrophages and neutrophils had reduced levels of cellular ATP (15–40% of wild-type levels), highlighting the crucial role of the transcription factor for energy generation through glycolysis in these immune cells<sup>15</sup>. Evidence of HIF activation in the diseased tissues of patients with inflammatory disorders such as rheumatoid arthritis<sup>16, 17</sup>, dermatomyositis<sup>18</sup>, congenital lupus<sup>19</sup> and atherosclerosis<sup>20</sup>, corroborates an important role for the hypoxic response in various human immunopathologies.

### HIF control of myeloid cell defense functions

Neutrophils and macrophages are not only participants in acute and chronic inflammatory pathologies, but they are also crucial front line effectors of innate host defense against invading microbial pathogens. While the oxygen tension in healthy tissues ranges from 17.5–63 mm Hg (that is, 2.5–9% oxygen), drastically lower levels (<1% oxygen) are characteristic of wounds and sites of infection (see Text Box). Thus phagocytic cells must be adapted to generate energy and function effectively in oxygen-deprived conditions, especially as many common bacterial pathogens proliferate readily in anaerobic microenvironments. Analysis of the bactericidal capacities of phagocytes from *Hif1a* conditional knockout mice validated a pivotal role for the hypoxic response in innate host defense. Compared to wild-type macrophages, macrophages isolated from mice deficient in HIF1 $\alpha$  are impaired in their capacity to kill Gram-positive and Gram-negative bacteria<sup>15, 21</sup>. When challenged subcutaneously with the pathogen group A streptococcus, myeloid cell *Hif1a* conditional knockout mice developed significantly larger necrotic ulcers and had higher bacterial loads in the infected tissue and blood<sup>21</sup>.

Analysis of the host–pathogen encounter by several groups has revealed several aspects of myeloid cell function that depend on HIF. In neutrophils, HIF induces leukocyte  $\beta 2$  integrin expression and thereby promotes neutrophil binding to epithelium<sup>22</sup>. HIF increases neutrophil expression of antimicrobial molecules such as cathelicidin peptides[G] and the granule proteases cathepsin G and elastase<sup>21</sup>. HIF also extends the functional neutrophil lifespan by inhibiting apoptotic pathways<sup>23, 24</sup>. Increased levels of HIF are also evident during the differentiation of blood monocytes into tissue macrophages<sup>25</sup>. HIF activity has been described as a key aspect of phagocytic uptake of bacteria by macrophages under hypoxic conditions<sup>26</sup>, and macrophage production of tumour necrosis factor (TNF) and nitric oxide (NO) through inducible NO synthetase (iNOS) is HIF dependent<sup>21</sup>. Since NO can stabilize HIF1 $\alpha$  protein by redistributing intracellular oxygen and inhibiting prolyl hydroxylase activity<sup>27</sup>, an autocrine feedback loop to amplify macrophage inflammatory activation is established. HIF-responsive elements are also found in the genes for Toll-like receptors (TLRs), including TLR2 and TLR6, which are upregulated in response to hypoxia<sup>28</sup>. Finally, HIF markedly increases the release of proinflammatory cytokines and the expression of co-stimulatory molecules by murine dendritic cells (DCs), enhancing their ability to induce allogeneic lymphocyte proliferation, thus helping to bridge innate and adaptive immune responses<sup>29</sup>.

One notable exception to the HIF-1 regulation of phagocyte function may be the generation of superoxide by the oxidative burst, which appears to occur with equal efficiency in wild-type and HIF 1 $\alpha$ -deficient macrophages<sup>21</sup>.

### Synergism of the hypoxic and innate immune responses

A surprising consequence of the findings summarized above is that, because of HIF activation, myeloid cells phagocytose and kill bacteria better under hypoxic conditions than they do under normoxic conditions<sup>21, 26</sup>. Strikingly, bacteria are an even stronger stimulus for HIF protein stabilization than is hypoxia itself, and bacteria-induced HIF protein stabilization can be readily demonstrated under normoxia<sup>21</sup>. Recently, the mechanistic explanation for these phenomena has been found to reflect a close, synergistic relationship between HIF and the central regulator of innate immunity, the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B).

NF- $\kappa$ B activity is controlled by I $\kappa$ B kinases (IKKs), mainly IKK $\beta$ , which carry out the phosphorylation-dependent degradation of I $\kappa$ B inhibitors in response to infectious or inflammatory stimuli<sup>30</sup>. HIF was shown to mediate NF- $\kappa$ B activation in anoxic neutrophils<sup>23</sup> and to promote the expression of NF- $\kappa$ B regulated cytokines in macrophages stimulated by lipopolysaccharide (LPS) in a TLR4-dependent manner<sup>31</sup>. Interestingly, hypoxia itself can stimulate NF- $\kappa$ B activation by inhibiting prolyl hydroxylases that negatively modulate IKK $\beta$  catalytic activity<sup>32</sup>.

NF- $\kappa$ B was found to contribute to increased *Hif1a* mRNA transcription under hypoxic conditions<sup>33, 34</sup>. The activation of HIF transcription by bacteria or LPS under normoxic as well as hypoxic conditions has been recently verified in a study using mice deficient in IKK $\beta$ <sup>35</sup>. Macrophages infected with Gram-positive or Gram-negative bacteria, or mice subjected to hypoxia, reveal a marked defect in HIF1 $\alpha$  expression following deletion of the

gene encoding IKK $\beta$ <sup>35</sup>. These results confirm that transcriptional activation of *Hif1a* by IKK $\beta$ -responsive NF- $\kappa$ B is a crucial precursor to post-transcriptional stabilization and accumulation of HIF1 $\alpha$  protein.

Because circulating phagocytes have a unique immune defense function, and must transit through different microenvironments during rapid mobilization to infected tissues, the synergistic HIF-NF $\kappa$ B pathway represents an elegant control mechanism for the specialized activities of these cells (Figure 2). Phagocyte bactericidal and pro-inflammatory capacities can be maintained in an “off” state while the myeloid cells circulate in the oxygen-rich blood; but then be activated in response to the decreasing oxygen gradient that is encountered following endothelial transcytosis and entry into the infected tissues. The primed phagocyte then undergoes a more potent stimulation of HIF pathway on direct pathogen encounter and, via recognition of pathogen-associated molecular patterns by TLRs, the activation of NF- $\kappa$ B and upregulation of *Hif1a* mRNA. The maximal “on-state” of bactericidal capacities is potentiated by the NO-mediated amplification loop, release of pro-inflammatory cytokines and upregulation of TLRs. This elegant control system links HIF to the myeloid cell response, ensuring that proinflammatory mediators, degradative enzymes and antimicrobial peptides are expressed preferentially in tissue site of infection, but not in healthy tissues where they would cause unwanted damage to host cells.

### Role of HIF in other cell types

Beyond the functions of myeloid cells including neutrophils, macrophages and DCs, epithelial cells and other specialized leukocytes also have important roles in innate host defense. Evidence is mounting showing that HIF is a key regulator of the intrinsic immune and inflammatory responses in a variety of non-myeloid cell types. For example, the skin provides a highly effective physical, cellular and chemical barrier against microbial penetration<sup>36</sup>. In response to bacterial pathogens, keratinocytes produce peptides of the cathelicidin and  $\beta$ -defensin family that can directly kill microbial pathogens<sup>37</sup>. HIF is expressed at high levels in the skin, which is a hypoxic organ even at baseline. Following targeted deletion of *Hif1a* in keratinocytes, mice show a defect in controlling necrotic skin infection by group A streptococcus<sup>38</sup>. Specific RNA interference studies confirmed that HIF-mediated regulation of keratinocyte cathelicidin production is crucial for cutaneous defense against infection with this invasive bacterium<sup>38</sup>.

HIF expression is upregulated in the gut epithelium during ischemia–reperfusion injury[G], and the level of HIF activation during the reperfusion phase is strongly increased by exposure to LPS or the bacterium *Pseudomonas aeruginosa*<sup>39</sup>. Targeted manipulation of HIF1 $\alpha$  expression in colonic epithelium also revealed an important role in the regulation of mucosal inflammatory responses to the irritant dextran-sulphate sodium (DSS). Mice with intestinal-specific disruption of HIF1 $\beta$  were protected against DSS-induced colitis, whereas those with constitutive HIF expression (as a result of VHL deletion) had increased expression of pro-inflammatory mediators, including macrophage migration inhibitor factor 1 (MIF1), leading to markedly increased oedema and cellular infiltrates<sup>40</sup>.

Mast cells are specialized granulocytic cells resident in the skin and the mucosa of the respiratory and gastrointestinal tracts. Well-studied for their roles in allergy, mast cells are increasingly recognized to function in both innate and adaptive immune responses<sup>41</sup>. Activation of HIF in human mast cells leads to release of pro-inflammatory cytokines such as interleukin-8 (IL-8) and TNF<sup>42</sup>. HIF activation in mast cells of the bronchial epithelium stimulates VEGF expression, leading to increased vascular permeability, protein extravasation into the alveolar space, and the hallmark airway oedema of asthma<sup>43</sup>. Moreover, HIF activation stimulates histidine decarboxylase expression by human mast cells, catalysing the formation of histamine, a potent inflammatory mediator<sup>44</sup>.

## HIF inflammatory responses during sepsis

Sepsis, the leading cause of death in intensive care units, reflects an aberrant and potentially lethal host response to overwhelming infection, in which bacteria or LPS provoke uncontrolled pro-inflammatory cytokine release from immune cells including monocytes and macrophages. LPS raises HIF levels in macrophages through activities of the mitogen-activated protein kinase (MAPK) and NF- $\kappa$ B signal transduction pathways<sup>33, 35</sup>, leading to increased levels of *Hif1a* mRNA transcripts, which are accompanied by a TLR4-dependent decrease levels of mRNA encoding prolyl hydroxylases<sup>31</sup>. Studies of LPS challenge of mice with HIF 1 $\alpha$ -deficient myeloid cells identified HIF as a crucial determinant of the sepsis phenotype, promoting high-level release of the pro-inflammatory cytokines TNF, IL-1 and IL-12. As a consequence, *Hif1a* deletion in the macrophage lineage protects animals against LPS-induced mortality and blocks the clinical manifestations of sepsis including hypotension, tachycardia and hypothermia<sup>31</sup>.

HIF transcriptional regulation also modulates pro-inflammatory cytokine production by CD4<sup>+</sup> and CD8<sup>+</sup> T cells, but in contrast to findings in myeloid cells, epithelial cells and mast cells, its net effect may be inhibitory. For example, following T cell receptor activation, the release of TNF and interferon- $\gamma$  (IFN $\gamma$ ) by T cells with targeted deletion of *Hif1a* was higher than wild-type cells<sup>45</sup>. The activated HIF-deficient T cells showed enhanced proliferation and contributed to blocking bacterial proliferation in a caecal ligation and puncture[G] model of sepsis<sup>46</sup>. The paradoxical behaviour of HIF in activated T cells may derive from alternative splicing and expression of a novel isoform of HIF1 $\alpha$  that inhibits cellular activation in a delayed feedback manner<sup>47</sup>. Data from studies involving the deletion of *Hif1a* specifically in T cells indicates that tight regulation of HIF function in these cells is crucial to prevent cell death, and implies that T cells may have a minor role in hypoxic tissues due to hypoxia-mediated and HIF-dependent cell death<sup>48</sup>.

## Dynamics of the HIF response to infectious pathogens

### Viruses

Acute infection with viruses is generally found to induce HIF protein stabilization in target cells, thus provoking local inflammation. For example, the common respiratory syncytial virus (RSV) induces HIF expression by human bronchial epithelial cells through a NO-dependent pathway, stimulating VEGF production and airway oedema characteristic of acute RSV infection<sup>49</sup>. In some cases, the HIF-dependent innate immune response to viral



infection may help to mitigate cytolytic injury and viral replication. Vesicular stomatitis virus (VSV) infection is an acute illness resulting in mouth ulcers in cattle and occasionally in humans. HIF activation by hypoxia or pharmacological agents can increase the expression of IFN $\beta$  and other antiviral genes and promote cellular resistance to VSV infection<sup>50</sup>.

By contrast, with certain persistent viral infections, the induction of HIF fails to result in eradication of the virus. An unfortunate consequence of HIF activation in these circumstances is that increased VEGF and the accompanying pro-angiogenic programme can contribute to oncogenic transformation. This seems to be the case in chronic infections with the hepatitis B and C viruses (HBV and HCV), which are epidemiologically associated with development of hepatocellular carcinoma, a highly vascularized solid tumour. HIF levels are increased in liver cells transfected with the oncogenic X protein of HBV (HBx) or in the livers of HBx-transgenic mice<sup>51</sup>. HBx interacts directly with HIF1 $\alpha$  to block its association with VHL and degradation by the ubiquitin–proteasome pathway<sup>52</sup>. Recently, HCV infection has also been found to stabilize HIF1 $\alpha$  protein with involvement of the NF- $\kappa$ B and MAPK signalling pathways, stimulating VEGF production and neovascularization<sup>53</sup>.

Human papillomavirus 16 (HPV-16) is an aetiological agent of cervical interstitial neoplasia that, if undetected, can progress to cervical carcinoma. Transfection of human cervical cells with the HPV-16 oncoproteins E6 and E7 induces VEGF expression and capillary formation in a HIF-dependent manner<sup>54</sup>, and HPV-16 and HIF1 $\alpha$  act synergistically to promote cancer lesions when expressed transgenically in the cervical epithelium of mice<sup>55</sup>. Increased HIF levels are correlated with poor prognosis of patients with advanced cervical cancer lesions, many of which are demonstrably hypoxic<sup>56</sup>. HIF activation is also apparent during other chronic viral infections that are associated with risk of neoplastic transformation, including human T-cell leukemia virus type I<sup>57</sup> and Epstein–Barr virus<sup>58, 59</sup>.

Crosstalk between viral genes and HIF activation has recently been shown to have an intriguing role in regulating latency and reactivation of human herpesvirus 8 (HHV-8). HHV-8 infection is associated with the endothelial tumour Kaposi's sarcoma in patients with AIDS. Biopsies of Kaposi's sarcoma lesions demonstrate high levels of HIF, and HHV-8 infection of endothelial cells can stabilize HIF1 $\alpha$  subunits and increase HIF target gene expression<sup>60</sup>. The HHV-8 latency-associated nuclear antigen stimulates *Hif1a* mRNA transcription and also physically interacts with the HIF heterodimer to enhance its promoter binding activities<sup>61</sup>. Induction and co-activation during HHV-8 infection allows HIF to bind HREs in the viral genome itself; these include one in the promoter of the gene encoding Rta, a key protein involved in reactivation of the virus from latency to a lytic replication phase<sup>61</sup>.

## Bacteria

Increased levels of HIF are observed in macrophages or neutrophils that are stimulated by a wide variety of bacterial species including groups A and B streptococci, *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*<sup>15, 21, 31, 62</sup>, indicating that HIF responds in a general manner to bacterial infection. The myeloid cell HIF response pathway is beneficial to innate immune defense, promoting bacterial killing *in vitro*<sup>15, 21, 31</sup> and restricting the spread of infection *in vivo*<sup>21</sup>. Bacterial products such as LPS and

peptidoglycan can activate TLRs and NF- $\kappa$ B signaling to increase transcription of HIF1 $\alpha$  {Frede, 2006 #108,<sup>35</sup>. Post-translational HIF1 $\alpha$  protein stabilization is provided by the exhaustion of oxygen at the tissue site of infection, and perhaps in certain cases, through iron sequestration by bacterial siderophores[G]. Indeed, siderophore-deficient *Yersinia enterocolitica* fail to induce HIF activation in intestinal Peyer's patches<sup>63</sup> and the virulence of *Y. enterocolitica* is increased in mice that lack intestinal HIF1 $\alpha$  expression, suggesting that the HIF response is important for mucosal innate defense.

*Bartonella henselae* is a facultative intracellular bacterium that causes an angioproliferative disorder known as bacillary angiomatosis in immunocompromised patients. bacillary angiomatosis lesions demonstrate high levels of HIF expression, and *B. henselae* activation of HIF-induced VEGF release is likely to provoke a pro-angiogenic programme that contributes to the characteristic tissue pathology<sup>64</sup>. Another intracellular bacterial pathogen, *Chlamydia pneumoniae*, has evolved a mechanism to counteract HIF protein stabilization, thereby inhibiting innate immune activation and promoting its survival in host cells. Secretion of a chlamydial protease-like activity factor into the cytoplasm degrades accumulated HIF1 $\alpha$ , facilitating continued *C. pneumoniae* replication during hypoxia<sup>65</sup>.

## Parasites

*Toxoplasma gondii* is an obligate intracellular parasite that produces opportunistic infections in fetuses and in immunocompromised individuals. *T. gondii* rapidly induces HIF expression by infected fibroblasts<sup>66</sup>, leading to upregulation of genes encoding glycolytic enzymes, glucose transporters and VEGF<sup>67</sup>. Under hypoxic conditions present in the tissues (brain, muscle and retina) in which the parasite produces serious clinical pathology, *T. gondii* replication and organelle maintenance was severely impaired in host cells that lack HIF1 $\alpha$ . It is thought that *T. gondii* has evolved to induce HIF expression because a particular HIF target gene is essential to parasite growth, or alternatively, because HIF activation is necessary to preserve the health of the host cell in which the parasite has become established<sup>66</sup>. Cutaneous lesions can be generated in BALB/c mice by infection with *Leishmania amazonensis*, and in the later stages of infection, HIF is induced in the cytoplasm and parasitophorous vacuoles of macrophages recruited to the skin lesions<sup>68</sup>.

## HIF-1 and SLC11A1 allele expression phenotypes

SLC11A1 (also known as Nramp1) is a protein-coupled divalent ion transporter involved in iron metabolism, resistance to pathogens, and inflammatory responses. *SLC11A1* was the first infectious disease susceptibility gene identified by positional cloning, and allelic variation in *SLC11A1* alters the risk for development of leishmaniasis<sup>69</sup> and tuberculosis<sup>70</sup>, as well as autoimmune conditions such as juvenile rheumatoid arthritis<sup>71</sup> and type 1 diabetes<sup>72</sup>. Interestingly, HIF differentially binds and activates Z-DNA-forming microsatellite polymorphisms[G] in the *SLC11A1* promoter region, thereby shaping allele expression phenotypes<sup>62</sup>, a newly described function for a transcription factor. Through its differential interaction with variant *SLC11A1* promoters, HIF transcriptional regulation has the potential to influence heritable differences in infectious and inflammatory disease susceptibility among and between human populations<sup>62</sup>.



## HIF: a pharmacological target to augment innate immunity

Given the accumulating evidence that HIF acts as a “master regulator” of the innate immune function of phagocytes<sup>73</sup>, it is possible that boosting HIF activity through pharmacological strategies might provide a novel approach to aid in the treatment of certain infectious disease conditions<sup>74</sup>. This concept is supported by the observation that macrophages that lack VHL, thus leading to constitutively high levels of HIF, are markedly more efficient than wild-type macrophages at killing Gram-positive and Gram-negative bacteria *in vitro*<sup>21</sup>. This implies that our phagocytic cells are not as good as they could be at killing bacteria, mainly because their activation is tightly regulated to limit unnecessary inflammatory injury. However, in the common clinical scenario of invasive bacterial infection, a failure of innate immunity occurs, and it is possible that such patients might benefit from augmentation of phagocytic cell bactericidal activity by HIF.

As proof-of-principle, addition of a series of pharmacological agonists of HIF, each a so-called “hypoxia mimetic” that restrict prolyl hydroxylase access to iron, directly enhanced murine macrophage bactericidal activity *in vitro*<sup>21</sup>. Similarly, a dose-dependent enhancement of the bactericidal activities of human whole blood, neutrophils and the macrophage cell line U937 against the pathogen *S. aureus* was achieved using one such hypoxia-mimetic, L-mimosine<sup>75</sup>. Local treatment with L-mimosine significantly delayed progression of *S. aureus* abscesses in a mouse subcutaneous challenge model<sup>75</sup>.

HIF agonists that are designed to activate phagocyte bactericidal mechanisms could conceivably be used alongside conventional antibiotics in localized infections, and would be predicted to function effectively against drug-resistant bacteria such as methicillin-resistant *S. aureus* (MRSA). The selective pressure for pathogens to evolve resistance to HIF agonist therapy may be negligible, since the drug targets the host and not the pathogen, deploying a multifaceted combination therapy of natural antimicrobial molecules<sup>76</sup>. However, an important cautionary note should be emphasized: based on evidence from studies of mice<sup>31</sup>, HIF agonists are probably inappropriate for systemic therapy of patients with disseminated infections and symptoms of sepsis, as macrophage pro-inflammatory cytokine and NO release could be precipitously increased and symptoms could worsen. Last, strategies to inhibit HIF for the treatment of chronic inflammatory disorders, such as rheumatoid arthritis, may provide a safer therapeutic margin than cytotoxic agents and high-dose steroids, as post-translational regulation of HIF1 $\alpha$  levels may allow rapid restoration of innate immune function of phagocytes following drug withdrawal in the event of opportunistic infection.

## Conclusions and Future Directions

A wealth of emerging information shows that HIF and the hypoxic response are deeply intercalated into the regulatory pathways of innate immune defence. The key implication of these findings is that the nature and magnitude of host bactericidal and inflammatory activities are highly dependent upon factors in the local tissue microenvironment, e.g. oxygen tension and iron availability, and cannot be simply extrapolated from *in vitro* model systems under ambient conditions. Through HIF control of immune cell energetics and gene expression pathways, antimicrobial activities can be focused and amplified where they are

needed most, namely tissue foci of infection, harsh and threatening microenvironments where oxygen and nutrients are limiting and cytotoxic molecules abound. We anticipate that future studies will reveal that major human pathogens modulate HIF activation pathways to subvert innate immunity or provoke dysregulated inflammation, contributing to cardinal clinical manifestations. A detailed understanding of the relationships between HIF, pathways of innate immune signal transduction such as TLRs/NF- $\kappa$ B, and the deployment of various immune effector molecules will provide a clearer and more physiologic understanding of infectious and inflammatory disease pathogenesis. Because of the short-half life and well understood mechanism for post-translational regulation of HIF levels, it represents an exciting pharmacologic target to fine tune immune cell functions up (antibiotic), or down (anti-inflammatory), in human disease therapeutics.

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## Glossary

<b>ubiquitin–proteasome pathway</b>	An important proteolytic pathway that involves the tagging of unwanted proteins with ubiquitin, which allows their recognition by the proteasome — a large, multi-component protein-degrading complex
<b>Cre recombinase</b>	Cre is a site-specific recombinase that recognizes and binds specific sites known as <i>loxP</i> . Two <i>loxP</i> sites recombine in the presence of Cre, allowing DNA that is cloned between two such sites to be removed by Cre-mediated recombination
<b>cathelicidin</b>	Mammalian cationic microbicidal peptides expressed by epithelial cells and phagocytes that share a highly conserved ‘cathelin’ 12 kDa pro-sequence at the amino terminus, followed by diversified mature sequences at the carboxy terminus. Activation of most cathelicidin precursors requires proteolytic cleavage to release the C-terminal domain, which has microbicidal and immunomodulatory activities
<b>ischaemia-reperfusion injury</b>	An injury in which the tissue first suffers from hypoxia as a result of severely decreased, or completely arrested, blood flow. Restoration of normal blood flow then triggers inflammation, which exacerbates the tissue damage
<b>caecal ligation and puncture</b>	An experimental model of peritonitis in rodents, in which the caecum is ligated and then punctured, thereby forming a small hole. This leads to leakage of intestinal bacteria into the peritoneal cavity and subsequent peritoneal infection
<b>siderophores</b>	Low molecular-weight compounds that are secreted by numerous types of bacteria and that have a high affinity for iron and other metal ions. These molecules chelate metal ions and carry them into

**Z-DNA-forming  
microsatellite  
polymorphisms**

the cell through specific receptors, promoting bacterial survival in the host

Microsatellites such as the SLC11A1 (GT/CA)<sub>n</sub> dinucleotide repeat have a propensity to form Z-DNA, an unstable left-handed form of DNA transiently induced during gene transcription by a moving RNA polymerase, and stabilized by negative supercoiling. Polymorphisms in such microsatellites have been shown to either activate or repress gene transcription in a context-dependent manner

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**Text Box****Normoxia**

Generally defined as either:

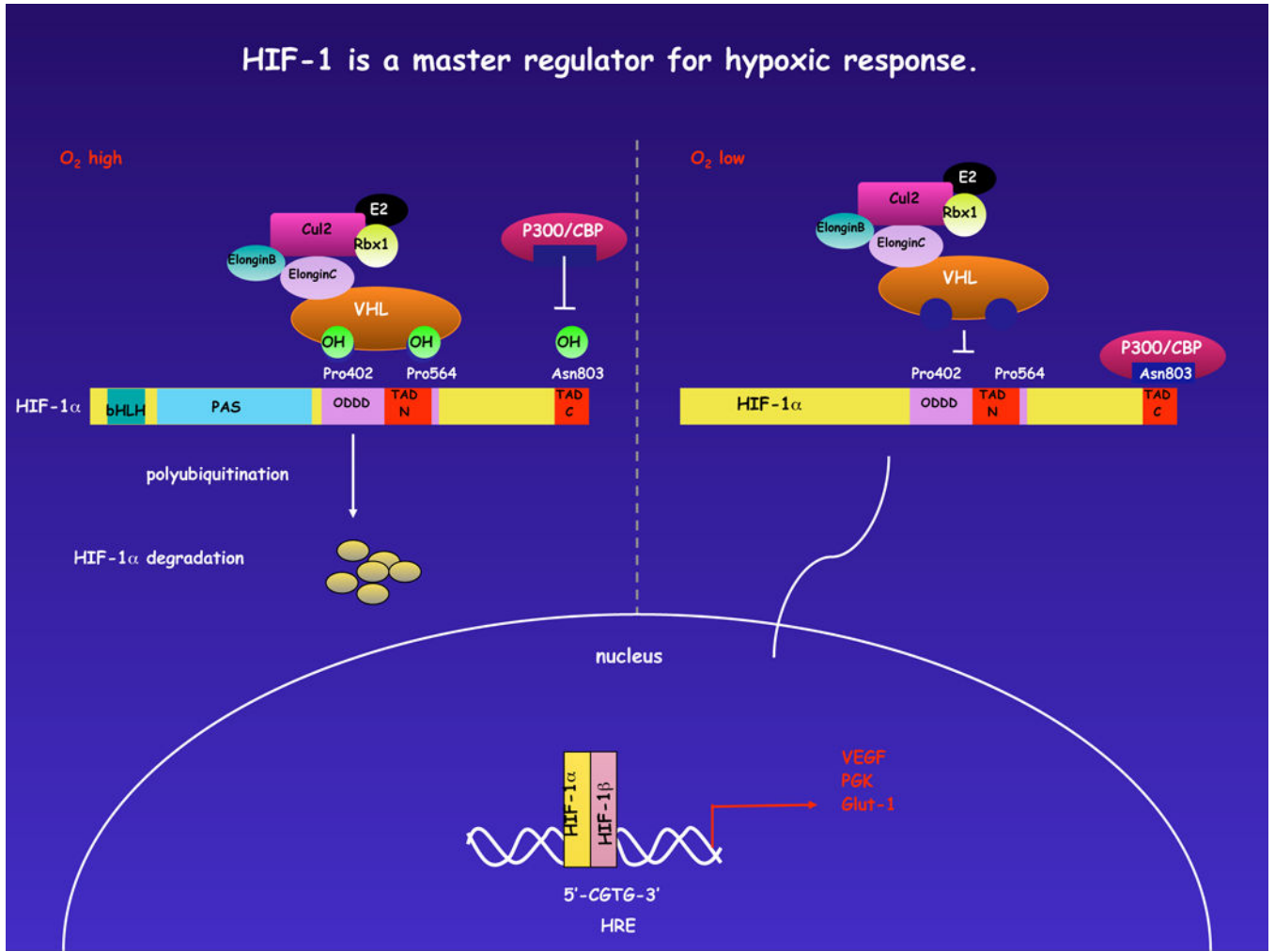
- a. atmospheric oxygen at sea level for tissue culture; or as
- b. physiological oxygenation in a well-vascularized and perfused tissue. This latter is, of course, dependent on the tissue in question, and in the case of innate immune system cells it becomes an even more elusive quantity to define.

**Hypoxia**

In tissue culture, generally defined as levels that are equivalent to between 0.5% – 3% oxygen by volume in the air that perfuses the growth medium. For actual tissues *in vivo*, this is more difficult to define, but is generally thought to occur in any tissue where injury or other alteration in perfusion causes a significant drop in tissue oxygen levels relative to those extant normally.

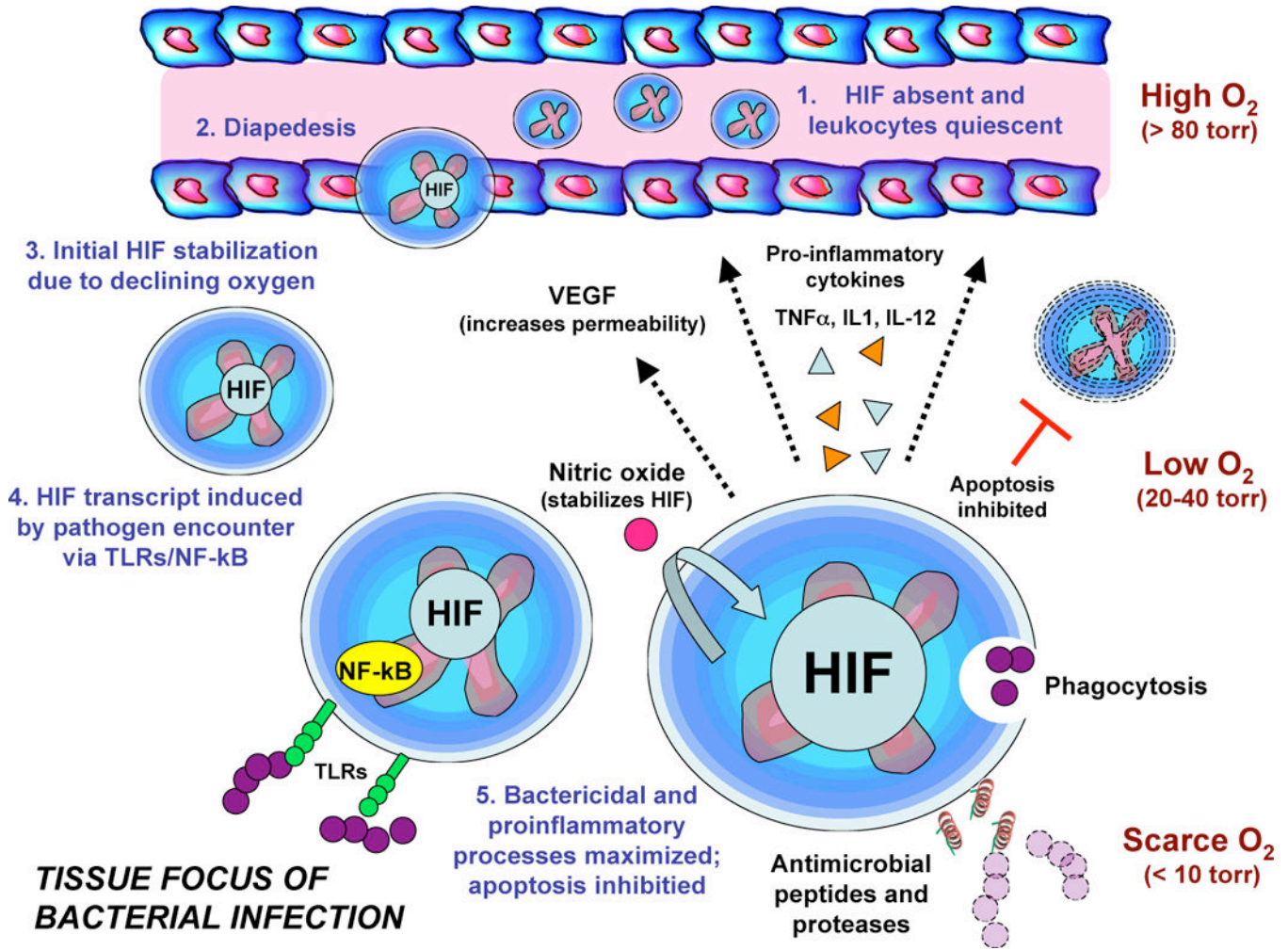
**Anoxia**

Defined in both tissue culture and physiology as the absence of physiologically available oxygen. Can occur in tissues in areas of acute infection and severe tissue damage.



**Figure 1. Mechanisms of HIF stabilization**

The current picture of post-translational regulation of the HIF response: Under normoxic conditions, hydroxylation by prolyl hydroxylases in an O<sub>2</sub>-dependent manner at the aa residues 402 and 564 acts to allow recognition/binding of HIF by the VHL complex which assembles in a classical SCF-like fashion around cullin-2, elongins B and C, and other elements of ubiquitination machinery. This results in a polyubiquitination of the HIF protein and its ultimate destruction by proteasomes. The asparagine hydroxylase FIH acts more or less in concert with the prolyl hydroxylation, although in this case to hydroxylate an asparagine residue in the C-terminal domain of HIF. This acts to block association with the p300/CBP protein; this in turn inhibits transcriptional enhancement by the HIF complex. As all of these post-translational events are dependent on intracellular oxygen, they are inhibited by hypoxia, and thus activation and transcription of HIF target genes facilitated when the various hydroxylations fail to occur, as depicted on the right.



**Figure 2. HIF regulation of phagocyte innate immune functions**

Myeloid-derived phagocytes such as neutrophils and macrophages have low hypoxia-inducible factor (HIF) levels at baseline as they circulate in the oxygen-rich blood. When recruited to tissue sites of infection, they migrate across the endothelium and immediately encounter a decreasing oxygen gradient, which leads to reduced prolyl hydroxylase activity and increased stabilization of HIF1α protein, which translocates to the nucleus and forms a functional heterodimeric transcription factor with HIF1β. Expression of innate immune response genes that contain hypoxia-responsive elements (HREs) in their promoters is increased, but maximal activation occurs only through Toll-like receptors (TLRs) and nuclear factor-κB (NF-κB) activation following pathogen encounter, which functions to boost HIF1α transcription. HIF activity promotes phagocytosis, inhibits apoptosis to increase phagocyte lifespan, stimulates the release of antimicrobial peptides, granule proteases, and pro-inflammatory cytokines such as tumour necrosis factor (TNF), interleukin-1 (IL-1) and IL-12, upregulates TLR expression, and activates the production of nitric oxide (NO) via inducible NO synthase (iNOS). NO interferes with HIF degradation, thereby creating an amplification loop for rapid phagocyte activation.

**Table 1**

Effects of infectious pathogens on HIF levels and contribution to disease pathogenesis

Name of Pathogen	Effect on HIF stabilization	Effect of altered HIF on disease pathogenesis	References
<b>Bacteria</b>			
<i>Streptococcus pyogenes</i>	Increases	Activation of phagocyte killing	31
<i>Streptococcus agalactiae</i>	Increases	Activation of phagocyte killing	15
<i>Pseudomonas aeruginosa</i>	Increases	Activation of phagocyte killing	31
<i>Salmonella typhimurium</i>	Increases	Activation of phagocyte killing	62
<i>Bartonella henselae</i>	Increases	Angiogenesis, vascular lesions	64
<i>Chlamydia pneumoniae</i>	Decreases	Promotes intracellular survival	65
<b>Bacterial components</b>			
Lipopolysaccharide	Increases	Phagocyte activation, but with high systemic doses, cytokine storm and sepsis syndrome	31, 34
Siderophores	Increases	Activation of mucosal defenses	63
<b>Viruses</b>			
Respiratory syncytial virus	Increases	VEGF release, airway oedema	49
Vesicular stomatitis virus	Increases	Interferon production and cellular resistance to virus	50
Hepatitis B virus	Increases	Neovascularization, oncogenesis	51
Hepatitis C virus	Increases	Neovascularization, oncogenesis	53
Human papillomavirus	Increases	Neovascularization, oncogenesis	54, 55
Human herpesvirus 8	Increases	Reactivation of virus from latency	60, 61
<b>Parasites</b>			
<i>Toxoplasma gondii</i>	Increases	Preserves viability of host cell in which pathogen is established	66
<i>Leishmania amazonensis</i>	Increases	Development of skin ulcers	68