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HYPOXIA AND INNATE IMMUNITY

Adapting to low oxygen levels

Rho family GTPases

Integrating signals for lymphocyte development and activation

Interdependence of hypoxic and innate immune responses

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Abstract | Hypoxia-inducible factor (HIF) is an important transcriptional regulator of cell metabolism and the adaptation to cellular stress caused by oxygen deficiency (hypoxia). Phagocytic cells have an essential role in innate immune defence 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- κ 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, such that the tissue microenvironment fundamentally influences susceptibility to infectious disease.

Acute foci of tissue 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^+ 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^{1,2}. In healthy tissues the oxygen tension is generally 20–70 mm Hg (that is, 2.5–9% oxygen), whereas markedly lower levels (<1% oxygen) have been described in wounds and necrotic tissue sites^{3–5}. The extreme local hypoxia is a consequence of decreased perfusion, which is secondary to microvascular injury, thrombosis or increased interstitial pressure, coupled with the metabolic activities of the infectious pathogen and the recruited inflammatory cells.

Myeloid cells are short-lived cells that are rapidly mobilized in response to any change in tissue integrity or entry of pathogenic microorganisms. They carry out phagocytosis of invading microorganisms and tissue debris and release a diverse array of antimicrobial molecules and pro-inflammatory mediators. Neutrophils and macrophages are crucial components of innate immune defence, serving to localize and eradicate pathogens and prevent the systemic spread of infection. In particular, neutrophil priming and apoptosis are crucial to the onset and resolution of granulocytic inflammation.

Deficiencies in the numbers of these specialized phagocytic cells (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 under normal conditions to avoid unwanted inflammatory injury to host tissues, but 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 preformed antimicrobial effectors following recognition of pathogens or cytokines. The field is gaining an improved understanding of how another key 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 front line innate immune effector cells have evolved to generate energy and carry out their microbicidal functions under hypoxic conditions. From these fundamental functions emerges an interdependence of the innate immune response and the hypoxic response, revealing a central role for the hypoxia-inducible factors (HIFs) as regulators of mammalian innate defence.

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Ubiquitin–proteasome pathway

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.

HIF: a central regulator of the hypoxic response

The HIF protein complex was originally discovered through its contribution to the marked induction of erythropoietin (*EPO*) gene transcription under hypoxic conditions^{6,7}. Further characterization showed that HIF is a heterodimeric helix–loop–helix transcription factor, the expression of which is regulated by both oxygen and iron (FIG. 1). HIF is active in all mammalian cells and is now known to regulate the expression of more than 100 genes that function in various host cellular and systemic responses to stress triggered by low oxygen levels. HIF-controlled pathways influence metabolism, angiogenesis, vascular tone, cell differentiation and apoptosis, and have implications for normal physiology and development but also for cancer and many other pathological conditions^{8,9}.

The HIF DNA-binding complex is comprised of the constitutively expressed *HIF1β* (also known as ARNT) subunit, which partners with one of two hypoxia-inducible α -subunits, *HIF1α* or *HIF2α* (also known as EPAS1)⁷. The HIF α -subunits are unstable under normoxic conditions as cells continually synthesize and degrade them. The half-lives of *HIF1α* and *HIF2α* are short owing to the activities of a family of oxygen- and iron-dependent prolyl hydroxylases (PHD1, PHD2 and PHD3)¹⁰, the actions of which direct HIF α -subunits for degradation by the ubiquitin–proteasome pathway in a process that depends on interaction with von Hippel–Lindau tumour suppressor protein (VHL)¹¹. A further level of control is provided by another hydroxylase, the asparaginyl hydroxylase factor inhibiting HIF (*FIH*; also known as *HIF1AN*), which has been shown to

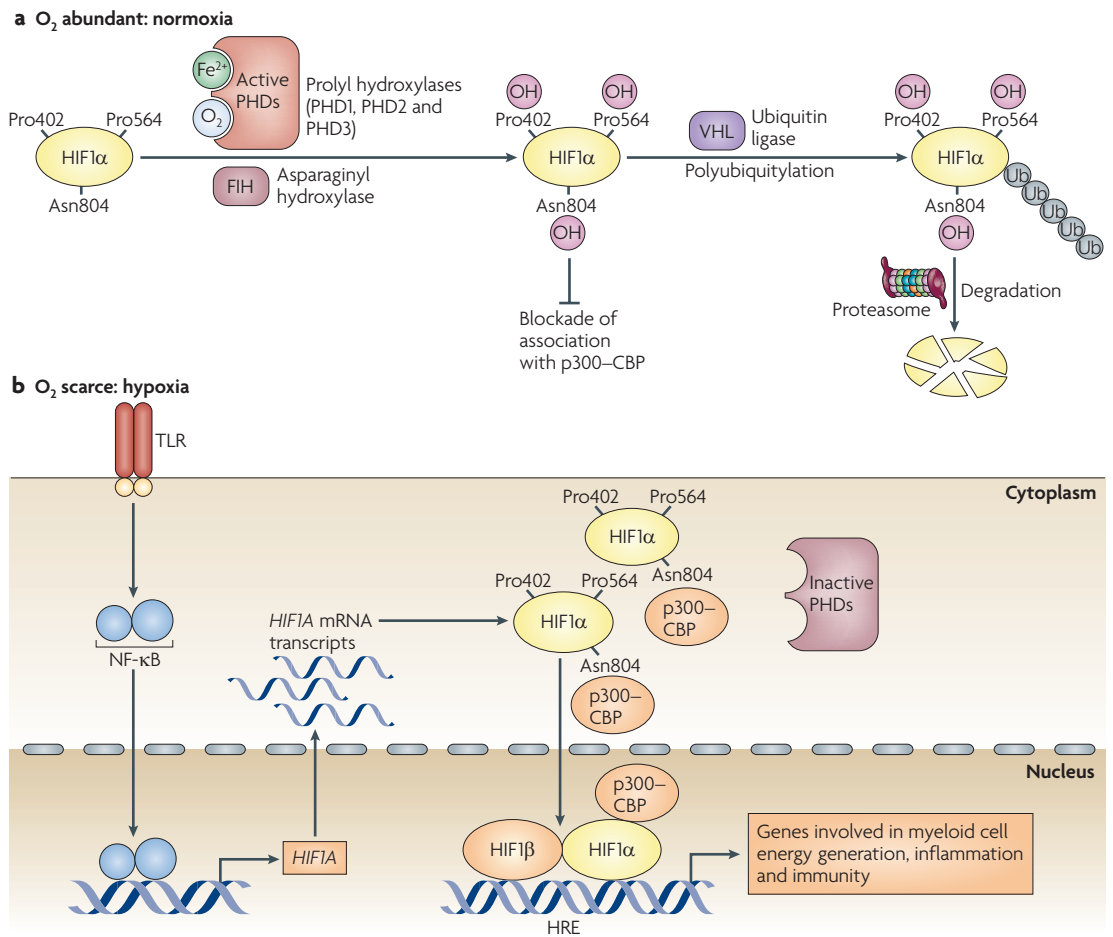


Figure 1 | Mechanisms of HIF stabilization. a | Under normoxic conditions, hydroxylation of hypoxia-inducible factor 1 α (*HIF1α*) by prolyl hydroxylases (PHDs) occurs in an O₂-dependent manner at amino acid residues 402 and 564. This results in polyubiquitylation of the *HIF1α* protein by von Hippel–Lindau tumour suppressor protein (VHL) and ultimately in the degradation of *HIF1α* by proteasomes. The asparaginyl hydroxylase factor inhibiting HIF (*FIH*; also known as *HIF1AN*) functions in conjunction with the prolyl hydroxylation, although in this case to hydroxylate an asparagine residue in the carboxy-terminal domain of *HIF1α*. This blocks the association of *HIF1α* with p300–CREB-binding protein (CBP; also known as CREBBP), which in turn inhibits transcriptional enhancement by the HIF complex (not shown). As all of these post-translational events depend on intracellular oxygen they are inhibited by oxygen deprivation. **b** | Under hypoxic conditions, *HIF1A* gene expression is upregulated through nuclear factor- κ B (NF- κ B) activation downstream of Toll-like receptors (TLRs), and the unmodified form of *HIF1α* in association with p300–CBP migrates to the nucleus to bind *HIF1β* (also known as ARNT), forming a heterodimeric helix–loop–helix transcriptional regulator. The HIF complex binds to target promoters known as hypoxic-response elements (HREs), leading to the transcription of genes that promote macrophage and neutrophil energy generation, inflammatory and bactericidal activities, and survival. Ub, ubiquitin.

Cre recombinase

A site-specific recombinase that recognizes and binds specific sites known as *LoxP*. Two *LoxP* sites recombine in the presence of Cre, allowing DNA that is cloned between two such sites to be removed by Cre-mediated recombination.

Cathelicidins

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 carboxyl terminus. Activation of most cathelicidin precursors requires proteolytic cleavage to release the C-terminal domain, which has microbicidal and immunomodulatory activities.

inhibit p300–CREB-binding protein (CBP; also known as CREBBP)-mediated transcriptional co-activation of the *HIF1A* genes^{12,13}.

Under conditions of hypoxia or iron deprivation, prolyl and asparaginyl hydroxylases are inhibited and HIF1 α or HIF2 α can accumulate and translocate to the nucleus, where they bind HIF1 β (FIG. 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 together to decrease mitochondrial oxygen consumption¹⁴, orchestrate the metabolic shift to anaerobic glycolysis¹⁵ and balance the decreased cellular pH owing to increased lactic acid production¹⁶, thereby 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. Indicative of the central roles of HIF1 α in development and physiology, deletion of HIF1 α in mice results in lethal embryonic defects in vascular development and morphology^{17–19}. Recently, a conditional gene targeting strategy exploiting Cre–*LoxP* recombination was used for lineage-specific elimination of HIF1 α in macrophages and neutrophils. A mouse line was engineered to contain *LoxP* sites flanking the *Hif1a* gene, and these mice were then crossed with mice in which the myeloid cell-specific lysozyme M promoter drives Cre recombinase expression. The resultant mice, which showed markedly decreased HIF1 α expression levels specifically in myeloid cells, have no obvious phenotypic abnormalities under normal conditions but showed marked aberrations in experimental models of acute inflammation²⁰. In contrast to their wild-type littermates, mice with a myeloid cell *Hif1a* conditional deletion 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, indicating impaired inflammatory responses²⁰. The HIF1 α -deficient macrophages and neutrophils had lower 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 cells²⁰. Evidence of HIF activation in the diseased tissues of patients with inflammatory disorders, such as rheumatoid arthritis^{21,22}, dermatomyositis²³, neonatal lupus syndrome²⁴ and atherosclerosis²⁵, suggests an important role for the hypoxic response in various human immunopathologies.

HIF control of myeloid cell defence functions.

Neutrophils and macrophages are not only participants in acute and chronic inflammatory pathologies but also crucial front line effectors of innate host defence against invading microbial pathogens. Although the oxygen percentage in healthy tissues ranges from 2.5–9% oxygen, markedly lower levels (<1% oxygen) are characteristic of wounds and sites of infection (BOX 1). 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 confirmed a pivotal role for the hypoxic response in innate host defence. Macrophages isolated from mice deficient in HIF1 α are impaired in their capacity to kill Gram-positive and Gram-negative bacteria compared with wild-type macrophages^{20,26}. When challenged subcutaneously with group A streptococcus, mice with a myeloid cell *Hif1a* conditional deletion developed significantly larger necrotic ulcers and had higher bacterial loads in the infected tissue and blood²⁶.

Analysis of the host–pathogen encounter by multiple groups has revealed several aspects of myeloid cell function that depend on HIF. In neutrophils, HIF induces β 2 integrin expression and thereby promotes neutrophil binding to the epithelium²⁷. HIF increases neutrophil expression of antimicrobial molecules such as cathelicidin peptides and the granule proteases cathepsin G and elastase²⁶. HIF also extends the lifespan of functional neutrophils by inhibiting apoptotic pathways^{28,29}. Increased levels of HIF are also evident during the differentiation of blood monocytes into tissue macrophages³⁰. HIF activity has been described as a key aspect of phagocytic uptake of bacteria by macrophages under hypoxic conditions³¹, and macrophage production of tumour necrosis factor (TNE) and synthesis of nitric oxide (NO) through inducible NO synthase (iNOS) is HIF dependent²⁶. HIF-responsive elements are also found in the genes encoding Toll-like receptors (TLRs), including TLR2 and TLR6, which are upregulated in response to hypoxia³². Finally, HIF markedly increases the release of pro-inflammatory cytokines and the expression of co-stimulatory molecules by murine dendritic cells (DCs), enhancing their ability to induce allogeneic lymphocyte proliferation and therefore helping to bridge innate and adaptive immune responses³³.

Box 1 | Defining oxygenation states**Normoxia**

Generally defined as either atmospheric oxygen at sea level for tissue culture or as physiological oxygenation in a well-vascularized and perfused tissue. Oxygenation of tissues depends on the tissue in question, and in the case of innate immune cells it becomes an even more elusive quantity to define.

Hypoxia

In tissue culture, hypoxia is generally defined as levels that are equivalent to between 0.5% and 3% oxygen by volume in the air that perfuses the growth medium. For actual tissues *in vivo* hypoxia is more difficult to define but is generally thought to occur in any tissue where injury or another alteration in perfusion causes a significant reduction in tissue oxygen levels relative to those that exist normally. Functionally, hypoxia exists *in vivo* whenever oxygen demand exceeds oxygen supply.

Anoxia

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

One notable exception to the HIF-mediated regulation of phagocyte function may be the generation of superoxides by the oxidative burst, which seems to occur with equal efficiency in wild-type and HIF1 α -deficient macrophages²⁶.

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^{26,31}. Strikingly, bacteria are an even stronger stimulus for HIF protein stabilization than is hypoxia itself, and bacteria-induced HIF protein stabilization can be readily shown under normoxia²⁶. Recently, the mechanistic explanation for these phenomena has been found to reflect a close, synergistic relationship between HIF and a central regulator of innate immunity, nuclear factor- κ B (NF- κ B) (a transcription factor).

NF- κ B activity is controlled by inhibitor of NF- κ B (I κ B) kinases (IKKs), mainly IKK β , which carry out the phosphorylation-dependent degradation of I κ B inhibitors in response to infectious or inflammatory stimuli³⁴. HIF was shown to mediate NF- κ B activation in neutrophils

under anoxic conditions²⁸ and to promote the expression of NF- κ B-regulated cytokines in macrophages stimulated by lipopolysaccharide (LPS) in a TLR4-dependent manner³⁵. Interestingly, hypoxia itself can stimulate NF- κ B activation by inhibiting prolyl hydroxylases that negatively modulate IKK β catalytic activity³⁶.

NF- κ B was found to contribute to increased *Hif1a* mRNA transcription under hypoxic conditions^{37,38}. The activation of *Hif1a* transcription by bacteria or LPS under normoxic as well as hypoxic conditions has been recently verified in a study using mice deficient in IKK β ³⁹. Macrophages infected with Gram-positive or Gram-negative bacteria, and mice subjected to hypoxia, reveal a marked defect in HIF1 α expression following deletion of the gene encoding IKK β ³⁹. These results confirm that transcriptional activation of *Hif1a* by IKK β -responsive NF- κ B is a crucial precursor to post-transcriptional stabilization and accumulation of HIF1 α protein.

Because circulating phagocytes have a unique immune defence function and must transit through different microenvironments during rapid mobilization to infected tissues, the synergistic HIF-NF- κ B pathway represents an elegant control mechanism for the specialized activities of these cells (FIG. 2). Phagocyte bactericidal

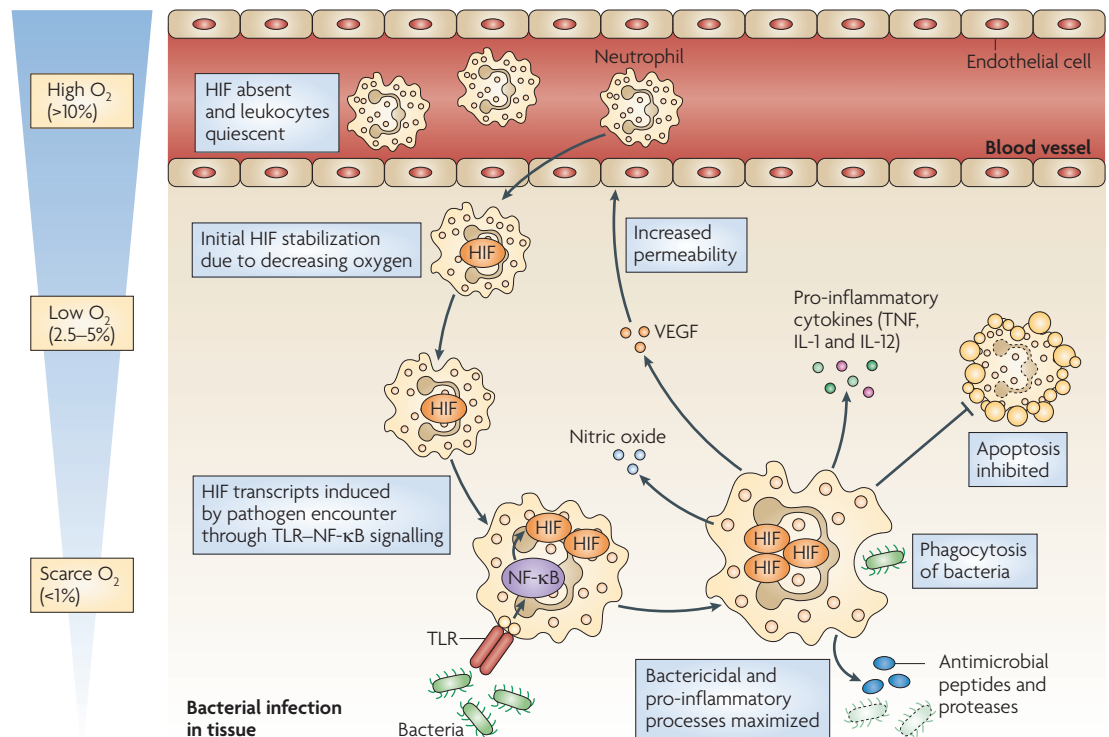


Figure 2 | HIF regulation of phagocyte innate immune functions. Myeloid-derived phagocytes such as neutrophils and macrophages have low levels of hypoxia-inducible factor (HIF) under normal conditions as they circulate in the oxygen-rich blood. When recruited to tissue sites of infection, they migrate across the endothelium and immediately encounter a decreasing oxygen gradient, which leads to decreased prolyl hydroxylase activity and increased stabilization of HIF1 α protein. HIF1 α translocates to the nucleus and forms a functional heterodimeric transcription factor with HIF1 β (also known as ARNT) (not shown). Expression of innate immune response genes that contain hypoxic-response elements (HREs) in their promoters is increased, but maximal activation occurs only through Toll-like receptors (TLRs) and nuclear factor- κ B (NF- κ B) activation following pathogen encounter, which functions to boost *HIF1A* transcription. HIF activity promotes phagocytosis; inhibits apoptosis to increase phagocyte lifespan; stimulates the release of antimicrobial peptides, granule proteases, vascular endothelial growth factor (VEGF, which increases vascular permeability) 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 by inducible nitric oxide synthase.

and pro-inflammatory capacities can be maintained in an 'off' state while the myeloid cells circulate in the oxygen-rich blood, but can 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 the HIF pathway on direct pathogen encounter and, through recognition of pathogen-associated molecular patterns by TLRs, activation of NF- κ B and upregulation of *Hif1a* mRNA. The maximal 'on state' of bactericidal capacities is enhanced by the release of pro-inflammatory cytokines and upregulation of TLR expression. This elegant control system links HIF to the myeloid cell response, ensuring that pro-inflammatory mediators, degradative enzymes and antimicrobial peptides are expressed preferentially at sites of infection, but not in healthy tissues where they would cause unwanted damage to host cells.

So far, functional studies of the HIF–NF- κ B pathway in myeloid cell innate immune functions have centred on the role of the HIF1 α subunit, but it will be important to expand these analyses to investigate the potential synergistic or modulatory roles of the HIF2 α subunit in this context. Periods of prolonged exposure to hypoxic conditions (18 hours) affect the expression of many macrophage cytokines and receptors, including interleukin-1 β (IL-1 β), IL-8, VEGF, *angiopoietin* and CXC-chemokine receptor 4 (CXCR4) in both mouse and human macrophages. In addition, recent studies using RNA interference and pharmacological inhibitors suggest that HIF1 α and HIF2 α , more so than NF- κ B, are important in orchestrating the changes in macrophage receptor and cytokine gene expression⁴⁰. Additional evidence suggests that HIF1 α , but not HIF2 α , underpins the synergistic induction of iNOS expression and other HRE-dependent transcriptional activities in macrophages following exposure to LPS and hypoxia⁴¹.

Role of HIF in other cell types

Evidence is mounting showing that HIF is a key regulator of the intrinsic immune and inflammatory responses in various non-myeloid cell types, including tissue epithelial cells and other specialized leukocytes. For example, the skin provides a highly effective physical, cellular and chemical barrier against microbial penetration⁴². In response to bacterial pathogens, keratinocytes produce peptides of the cathelicidin and β -defensin family that can directly kill microbial pathogens⁴³. HIF is expressed at high levels in the skin, which is a hypoxic organ even in non-inflammatory settings⁴⁴. Following targeted deletion of *Hif1a* in keratinocytes, mice show a defect in controlling necrotic skin infection by group A streptococci⁴⁴. Specific RNA interference studies confirmed that HIF1 α -mediated regulation of keratinocyte cathelicidin production is crucial for cutaneous defence against infection with this invasive pathogen⁴⁴.

HIF expression is upregulated in the gut epithelium during ischaemia–reperfusion injury, and the level of HIF activation during the reperfusion phase is strongly increased by exposure to LPS or the bacterium *Pseudomonas aeruginosa*⁴⁵. Targeted manipulation of HIF1 α expression

in colonic epithelium also suggests potential roles for HIF1 α in the regulation of intestinal mucosal inflammatory responses, although the directionality of the effects has been conflicting. In one study⁴⁶, mice with intestine-specific disruption of HIF1 α expression were protected against dextran sulphate sodium-induced colitis, whereas those with constitutive HIF expression (as a result of VHL deletion) had increased expression of pro-inflammatory mediators, including macrophage migration inhibitory factor (MIF), leading to markedly increased oedema and cellular infiltrates. However, several other studies using different mouse colitis models indicated the opposite: a protective effect of HIF against colonic inflammation following genetic manipulation⁴⁷ or pharmacological interventions^{48,49}.

Mast cells are specialized granulocytic cells that are resident in the skin and the mucosa of the respiratory and gastrointestinal tracts. Their roles in allergy are well studied and they are increasingly recognized to function in both innate and adaptive immune responses⁵⁰. Activation of HIF in human mast cells leads to release of pro-inflammatory cytokines such as IL-8 and TNF⁵¹. HIF activation in mast cells of the bronchial epithelium stimulates VEGF expression, leading to increased vascular permeability, protein extravasation into the alveolar space and airway oedema⁵². Moreover, HIF activation stimulates histidine decarboxylase expression by human mast cells, catalysing the formation of histamine, a potent inflammatory mediator⁵³. To date, the role of HIF in eosinophil inflammatory functions has not been examined directly.

HIF inflammatory responses during sepsis

Sepsis, the leading cause of death in intensive care units, is an aberrant and potentially lethal host response to overwhelming infection, in which bacteria or LPS provoke uncontrolled release of pro-inflammatory cytokines from immune cells, including monocytes and macrophages. LPS raises HIF levels in macrophages through activities of the mitogen-activated protein kinase (MAPK) and NF- κ B signal transduction pathways^{37,39}, leading to increased levels of *Hif1a* mRNA transcripts, which are accompanied by a TLR4-dependent decrease in the levels of mRNAs encoding prolyl hydroxylases³⁵. Studies of LPS challenge of mice with HIF1 α -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 hypothermia³⁵.

HIF transcriptional regulation also modulates pro-inflammatory cytokine production by CD4⁺ and CD8⁺ T cells but, in contrast to findings in myeloid cells, epithelial cells and mast cells, the net effect of T cell-produced HIF might be inhibitory. For example, following T cell receptor activation, the release of TNF and interferon- γ (IFN γ) by T cells with targeted deletion of *Hif1a* was higher than release by wild-type T cells⁵⁴. The activated HIF-deficient T cells showed enhanced proliferation

Ischaemia–reperfusion injury

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.

Caecal ligation and puncture

An experimental model of peritonitis in rodents, in which the caecum is ligated and then punctured. This leads to leakage of intestinal bacteria into the peritoneal cavity and subsequent peritoneal infection.

and contributed to blocking bacterial proliferation in a caecal ligation and puncture model of sepsis⁵⁵. The paradoxical behaviour of HIF in activated T cells may derive from alternative splicing and expression of an isoform of HIF1 α that inhibits cellular activation in a delayed-feedback manner⁵⁶. Data from studies involving the deletion of *Hif1a* specifically in T cells indicate that tight regulation of HIF function in these cells is crucial to prevent cell death, and imply that T cells may have a minor role in hypoxic tissues owing to hypoxia-mediated and HIF-dependent cell death⁵⁷.

HIF dynamics in response to infectious pathogens

Viruses. Acute infection with viruses is generally found to induce HIF protein stabilization in target cells (TABLE 1), 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 the airway oedema that is characteristic of acute RSV infection⁵⁸. 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 β and other antiviral genes and promote cellular resistance to VSV infection⁵⁹.

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 the development of hepatocellular carcinoma, a highly vascularized solid tumour. Experimental evidence of this includes the finding that HIF levels are increased in liver cells transfected with the oncogenic X protein of HBV (HBx) and in the livers of HBx-transgenic mice⁶⁰. HBx interacts directly with HIF1 α to block its association with VHL and degradation by the ubiquitin–proteasome pathway⁶¹. Recently, HCV infection has also been found to stabilize HIF1 α protein, with the involvement of the NF- κ B and MAPK signalling pathways, stimulating VEGF production and neovascularization⁶².

Human papilloma virus type 16 (HPV-16) is an aetiological agent of cervical interstitial neoplasia that, if untreated, 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 manner⁶³, and HPV-16 and HIF1 α act synergistically to promote cancer lesions when expressed transgenically in the cervical epithelium of mice⁶⁴. Increased HIF levels

Table 1 | **Effects of infectious pathogens on HIF levels and contribution to disease pathogenesis**

Pathogen	Effect on HIF stabilization	Effect of altered HIF on disease pathogenesis	Refs
Bacteria			
<i>Streptococcus pyogenes</i>	Increases	Activation of phagocyte killing	35
<i>Streptococcus agalactiae</i>	Increases	Activation of phagocyte killing	20
<i>Pseudomonas aeruginosa</i>	Increases	Activation of phagocyte killing	35
<i>Salmonella typhimurium</i>	Increases	Activation of phagocyte killing	71
<i>Bartonella henselae</i>	Increases	Angiogenesis and vascular lesions	73
<i>Chlamydia pneumoniae</i>	Decreases	Promotes intracellular survival of bacterium	74
Bacterial components			
Lipopolysaccharide	Increases	Phagocyte activation, but with high systemic doses, cytokine storm and sepsis syndrome occur	35,37,39
Siderophores	Increases	Activation of mucosal defences	72
Viruses			
Respiratory syncytial virus	Increases	VEGF release and airway oedema	58
Vesicular stomatitis virus	Increases	Type I interferon production and cellular resistance to virus	59
Hepatitis B virus	Increases	Neovascularization and oncogenesis	60
Hepatitis C virus	Increases	Neovascularization and oncogenesis	62
Human papilloma virus	Increases	Neovascularization and oncogenesis	63,64
Human herpesvirus 8	Increases	Reactivation of virus from latency	69,70
Parasites			
<i>Toxoplasma gondii</i>	Increases	Preserves viability of infected host cell	75
<i>Leishmania amazonensis</i>	Increases	Development of skin ulcers	77

HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor.

are correlated with poor prognosis of patients with advanced cervical cancer lesions, many of which are demonstrably hypoxic⁶⁵. HIF activation is also apparent during chronic infection with other viruses that are associated with the risk of neoplastic transformation, including human T cell leukaemia virus type I⁶⁶ and Epstein–Barr virus^{67,68}.

Crosstalk between viral genes and HIF activation has recently been shown to have an intriguing role in regulating the latency and reactivation of human herpesvirus 8 (HHV8). HHV8 infection is associated with the endothelial tumour Kaposi's sarcoma in patients with AIDS. Biopsies of Kaposi's sarcoma lesions show high levels of HIF, and HHV8 infection of endothelial cells can stabilize HIF1 α subunits and increase HIF target gene expression⁶⁹. The HHV8 latency-associated nuclear antigen stimulates *Hif1a* mRNA transcription and also physically interacts with the HIF heterodimer to enhance its promoter-binding activities⁷⁰. Induction and co-activation during HHV8 infection allow 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 phase⁷⁰.

Bacteria. Increased levels of HIF are observed in macrophages and neutrophils stimulated by various bacterial species, including group A and B streptococci, *Staphylococcus aureus*, *Salmonella typhimurium* and *P. aeruginosa*^{20,26,35,71}, indicating that HIF responds in a general manner to bacterial infection (TABLE 1). The myeloid cell HIF response pathway is beneficial to innate immune defence, promoting bacterial killing *in vitro*^{20,26,35} and restricting the spread of infection *in vivo*²⁶. Bacterial products such as LPS and peptidoglycan can activate TLRs and NF- κ B signalling to increase the transcription of *Hif1a*³⁷. Post-translational HIF1 α 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. Indeed, siderophore-deficient *Yersinia enterocolitica* fails to induce HIF activation in intestinal Peyer's patches⁷² and the virulence of *Y. enterocolitica* is increased in mice that lack intestinal HIF1 α expression, suggesting that the HIF response is important for mucosal innate defence.

Bartonella henselae is a facultative intracellular bacterium that causes an angioproliferative disorder known as bacillary angiomatosis in immunocompromised patients. Bacillary angiomatosis lesions show 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 pathology⁷³. 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 α , facilitating continued *C. pneumoniae* replication during hypoxia⁷⁴.

Parasites. *Toxoplasma gondii* is an obligate intracellular parasite that produces opportunistic infections in fetuses and in immunocompromised individuals. It rapidly induces HIF expression by infected fibroblasts⁷⁵, which leads to upregulation of genes encoding glycolytic enzymes, glucose transporters and VEGF⁷⁶. Under hypoxic conditions in the tissues (brain, muscle and retina) in which the parasite produces serious clinical pathology, *T. gondii* replication and organelle maintenance were severely impaired in host cells that lacked HIF1 α . It is thought that *T. gondii* has evolved to induce HIF expression because a particular HIF target gene is essential to parasite growth or because HIF activation is necessary to preserve the health of the host cell in which the parasite has become established⁷⁵. 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 lesions⁷⁷. Whether this observed HIF induction contributes to immune resolution of leishmanial infection is not yet understood.

HIF and SLC11A1 allele expression phenotypes. Solute-carrier family 11, member A1 (SLC11A1; also known as NRAMP1) is a proton-coupled divalent ion transporter that is involved in iron metabolism, resistance to pathogens and inflammatory responses. SLC11A1 was the first infectious disease susceptibility gene to be identified by positional cloning, and allelic variation in SLC11A1 alters the risk for development of leishmaniasis⁷⁸ and tuberculosis⁷⁹ as well as autoimmune conditions such as juvenile rheumatoid arthritis⁸⁰ and type 1 diabetes⁸¹. Interestingly, HIF differentially binds and activates Z-DNA-forming microsatellite polymorphisms in the SLC11A1 promoter region, thereby shaping allele expression phenotypes⁷¹, 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 within and between human populations⁷¹.

HIF: a drug target to boost innate immunity

Given the accumulating evidence that HIF functions as a 'master regulator' of the innate immune function of phagocytes⁸², it is possible that boosting HIF activity through pharmacological strategies might provide a new approach to aid the treatment of certain infectious disease conditions⁸³. This concept is supported by the observation that macrophages that lack VHL and thus have constitutively high levels of HIF are markedly more efficient than wild-type macrophages at killing Gram-positive and Gram-negative bacteria *in vitro*²⁶. This implies that normal phagocytic cells are not as adept at killing bacteria as they could be, mainly because their activation is tightly regulated to limit unnecessary inflammatory injury. However, the common clinical scenario of invasive bacterial infection is linked to a failure of innate immunity to control the infecting pathogen, 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 'hypoxia

Siderophores

Low-molecular-mass 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 the cell through specific receptors, promoting bacterial survival in the host.

Z-DNA-forming microsatellite polymorphisms

Microsatellites such as the SLC11A1 (GT/CA)_n dinucleotide repeat tend to form Z-DNA, an unstable left-handed form of DNA that is transiently induced during gene transcription by a moving RNA polymerase and stabilized by negative supercoiling. Polymorphisms in such microsatellites can activate or repress gene transcription in a context-dependent manner.

mimetic' that restricts prolyl hydroxylase access to iron, directly enhanced mouse macrophage bactericidal activity *in vitro*²⁶. 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-mimosine⁸⁴. Local treatment with L-mimosine significantly delayed progression of *S. aureus* abscesses in a mouse subcutaneous challenge model⁸⁴.

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 as the drug targets the host and not the pathogen, thereby deploying a multifaceted combination therapy of natural antimicrobial molecules⁸⁵. The effects of such drugs would not be restricted to immune cells. However, chronic administration of a prolyl hydroxylase inhibitor or HIF agonist to non-human primates was not associated with significant toxicity, and these compounds have advanced to Phase Ib–II clinical trials in over 700 patients for treatment of the anaemia associated with chronic kidney disease⁸⁶. An important cautionary note should be emphasized: based on evidence from studies of mice³⁵, HIF agonists are probably inappropriate for systemic therapy of patients that have disseminated infections and symptoms of sepsis, as macrophage pro-inflammatory cytokine and NO release could be rapidly increased and symptoms could worsen. Instead, one appealing starting point may be local or topical administration for treatment or prophylaxis of bacterial skin and wound infections, where fortification of the antimicrobial barrier provided by keratinocytes and myeloid cells could be coupled with the reported enhancement of cutaneous wound healing associated with HIF augmentation⁸⁷.

Finally, it is possible that 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 α 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 involved with 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 on factors in the local tissue microenvironment — for example, 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 foci of tissue infection, which are harsh and threatening microenvironments where oxygen and nutrients are limiting and cytotoxic molecules abound. We anticipate that future studies will reveal that important human pathogens modulate HIF activation pathways to subvert innate immunity or provoke dysregulated inflammation, contributing to key clinical manifestations. A detailed understanding of the relationships between HIF, pathways of innate immune signal transduction such as TLR–NF- κ B signalling and the deployment of various immune effector molecules will provide a clearer and more physiological understanding of infectious and inflammatory disease pathogenesis. Because of the short half-life and well-understood mechanism for post-translational regulation of HIF levels, HIF is an attractive pharmacological target to fine-tune immune cell functions for the treatment of human disease.

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Acknowledgements

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DATABASES

UniProtKB: <http://www.uniprot.org/angiotensinogen|CXCR4|EH1|HIF1a|HIF1b|HIF2a|JENy|IL-1b|IL-8|iNOS|MIF|SLC11A1|TNE|VEGF>

FURTHER INFORMATION

Randall S. Johnson's homepage:

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