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### Review Series

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# Hypoxia-inducible factors: key regulators of myeloid cells during inflammation

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Hypoxia is a prominent characteristic of many acute or chronic inflammatory diseases, and exerts significant influence on their progression. Macrophages and neutrophils are major cellular components of innate immunity and contribute not only to O<sub>2</sub> deprivation at the site of inflammation, but also alter many of their functions in response to hypoxia to either facilitate or suppress inflammation. Hypoxia stabilizes HIF- $\alpha$ s in macrophages and neutrophils, and these O<sub>2</sub>-sensitive transcription factors are key regulators of inflammatory responses in myeloid cells. In this review, we will summarize our current understanding of the role of HIF- $\alpha$ s in shaping macrophage and neutrophil functions in the pathogenesis and progression of multiple inflammatory diseases.

## Inflammation and hypoxia

Inflammation constitutes the body's defensive response to injury and/or infection in order to eliminate pathogens and damaged tissue, while initiating tissue repair and healing. Upon tissue injury or pathogen invasion, local sentinel cells such as resident macrophages and mast cells respond to dilate blood vessels, increase vascular permeability, and recruit a variety of leukocytes to the site of inflammation. During the acute phase of inflammatory responses, a major task of such recruited cells is the clearance of damaged tissue or pathogens. Upon transition into the resolution phase, tissue homeostasis is gradually restored. If acute inflammation fails to subside, it progresses into chronic inflammation with potentially serious consequences for the afflicted patient (1, 2). One feature of inflammation sites is low oxygen (O<sub>2</sub>) tension, termed "hypoxia." Oxygen tension ranges between 2.5% and 9% in most healthy tissues. However, poor O<sub>2</sub> availability resulting from damaged vasculature, high metabolic rates of bacteria and other pathogens, and numerous infiltrating immune cells deprive inflamed tissue of O<sub>2</sub>, frequently leading to partial O<sub>2</sub> pressures (pO<sub>2</sub>) of less than 1% (3, 4).

The interdependence between inflammation and hypoxia has been evident for many years. Hypoxia is prevalent in multiple inflammatory scenarios, such as inflammatory bowel diseases (IBDs) and rheumatoid arthritis (RA) (5–8). The intestinal mucosa exhibits an O<sub>2</sub> gradient from crypt to villus, wherein O<sub>2</sub> is highest in the crypts and lowest in the villus tips, which are closest to the anoxic gut lumen (9). This "physiological" hypoxia is largely extended with intestinal inflammation (7, 8). Hypoxia is also a characteristic of inflamed joints in patients with RA. Using a highly sensitive gold microelectrode, investigators accurately measured synovial O<sub>2</sub> tension in RA patients (5), demonstrating that RA median O<sub>2</sub> tension in synovial tissue (2%–4%) was much lower than that in the noninflamed synovium (9%–12%) (5, 6).

Low O<sub>2</sub> tension can also directly contribute to inflammation. In the setting of obesity, hypoxia develops as adipose tissue mass expands, initiating inflammatory responses. Secretion of inflammation-related adipokines (e.g., TNF- $\alpha$  and leptin) increases in hypoxic adipose tissue. Together with additional disruptions in glucose and lipid metabolism, this inflammation can become chronic and systemic, eventually leading to insulin resistance (10, 11). In the lung, alveolar hypoxia can be induced by exposing rats to 10% O<sub>2</sub> for up to 8 hours, which triggers macrophage recruitment, enhances expression of HIF-1 $\alpha$  and inflammatory cytokines (e.g., macrophage inflammatory protein 1- $\alpha$  [MIP-1 $\alpha$ ], monocyte chemoattractant protein-1 [MCP-1], and TNF- $\alpha$ ), promotes NF- $\kappa$ B activity, and elevates albumin leakage (12). Similar observations were made in mice exposed to 5% O<sub>2</sub> for 60 minutes, where levels of IL-6, TNF- $\alpha$ , and IL-1 $\alpha$  were elevated in serum and isolated peritoneal macrophages and Kupffer cells (liver macrophages) (13). In humans, hypoxia-induced inflammation is evident in individuals with high-altitude illness. Those who ascend rapidly are at risk of developing high-altitude pulmonary and cerebral edema, caused by hypoxic pulmonary vasoconstriction, high arterial and capillary pressure, and elevated levels of circulating IL-6, IL-1 receptor antagonist (IL-1RA), and C-reactive protein (CRP) (14, 15). All of these examples indicate that the relationship between inflammation and hypoxia exists in many pathological settings, and is a potentially attractive therapeutic target.

## Hypoxia and hypoxia-inducible factors

In many of the pathological situations described above (5, 7, 8, 12, 16, 17), HIFs are activated in response to the hypoxic and inflammatory microenvironment. HIFs represent the primary O<sub>2</sub>-sensing transcription factors (18–21) as heterodimers comprised of an O<sub>2</sub>-sensitive  $\alpha$  subunit (HIF- $\alpha$ ) and a constitutively expressed  $\beta$  subunit (HIF-1 $\beta$  or aryl hydrocarbon receptor nuclear translocator [ARNT]). Three  $\alpha$  subunits have been discovered thus far: HIF-1 $\alpha$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$ . While HIF-1 $\alpha$  and HIF-2 $\alpha$  are well characterized, relatively little is known about HIF-3 $\alpha$  (22, 23). The *HIF3A* gene encodes multiple HIF-3 $\alpha$  variants, which are struc-

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turally distinct from HIF-1 $\alpha$  and HIF-2 $\alpha$ , as they lack a C-terminal transactivation domain. Divergence in structure and variant diversity allow HIF-3 $\alpha$  to have numerous modes of action, regulating a transcriptional program that is distinct from that of HIF-1 $\alpha$  (24). In this review, we will focus on HIF-1 $\alpha$  and HIF-2 $\alpha$ , but additional details about HIF-3 $\alpha$  are reviewed elsewhere (23–25). Under normoxia, the O<sub>2</sub>-sensitive  $\alpha$  subunit is hydroxylated on two conserved proline residues (P402/P405 and P564/P531 for HIF-1 $\alpha$ /HIF-2 $\alpha$ , respectively) within the O<sub>2</sub>-dependent degradation domain (ODD) by prolyl hydroxylase domain-containing proteins (PHDs) (22, 26). Hydroxylated HIF- $\alpha$  subunits are then polyubiquitinated by the von Hippel-Lindau (VHL) tumor suppressor E3 ubiquitin ligase complex and subsequently degraded via the 26S proteasome (27–29). Under hypoxia, PHDs cannot hydroxylate key HIF- $\alpha$  proline residues due to limited access to their substrate (O<sub>2</sub>) or redox imbalance (21, 30–32), resulting in HIF- $\alpha$  stabilization. Stabilized HIF- $\alpha$ s translocate into the nucleus, dimerize with their obligate binding partner ARNT, recruit additional coactivators, and bind to hypoxia-response elements (HREs) to enhance transcription of hundreds of genes whose products mediate cellular adaptation to hypoxia. Such pathways include metabolism, angiogenesis, and inflammatory responses.

Other than O<sub>2</sub>-dependent HIF posttranslational modifications, HIF- $\alpha$  stabilization can be induced by inflammatory stimuli independently of hypoxia. The proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  promote HIF-1 $\alpha$  accumulation in an NF- $\kappa$ B-dependent manner (33–35). Bacterial products, such as LPS, can also stabilize HIF-1 $\alpha$  under normoxia through multiple pathways, such as NF- $\kappa$ B (36, 37), ROS (38), PHDs (39), and MAPKs (40). On the other hand, hypoxic responses can also be HIF independent. For example, hypoxia suppresses mTOR activity independently of HIF signaling (41), via the mTOR inhibitor REDD1 and the TSC1/TSC2 complex (42). Other hypoxia-responsive pathways include endoplasmic reticulum (ER) stress (43) and NF- $\kappa$ B (37, 44, 45) pathways. One myeloid-specific example is mentioned later in this review (46).

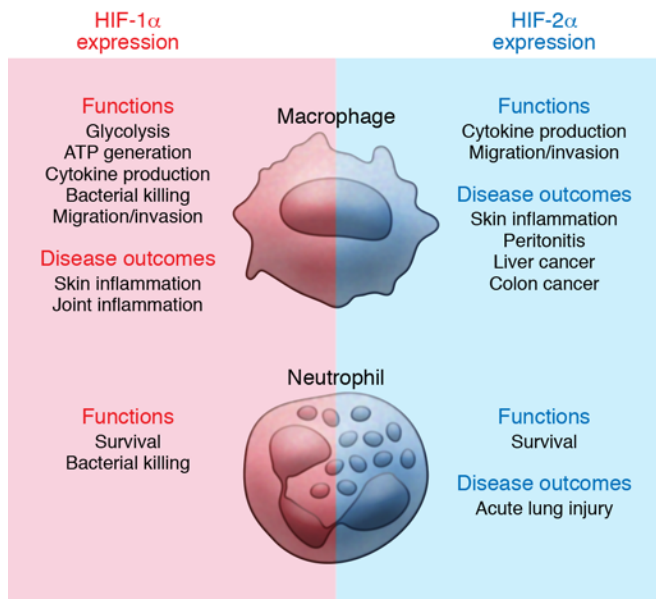
## Myeloid cells in inflammation

Macrophages are key cellular components of innate immunity and encompass a highly heterogeneous population of cells with a broad array of phenotypes and functions. Some of these cells are distributed over most of the body, residing in many tissues (e.g., Kupffer cells in the liver, osteoclasts in the bone, and microglia in the brain), while others are differentiated monocytes that infiltrate sites of inflammation to promote adaptive responses or facilitate restoration of tissue homeostasis (47, 48). Upon pathogen invasion or injury, tissue-resident macrophages represent the first responders, recruiting neutrophils via secretion of chemokines (e.g., IL-8 in humans and CXCL1 in mice). Once neutrophils arrive at the compromised site, they release monocyte chemoattractants (e.g., MCP-1) so that large numbers of recruited monocytes/macrophages extend the inflammatory response (49–51). These macrophages normally adopt a proinflammatory or “classically activated” (M1) phenotype, which is often induced by IFN- $\gamma$  and Toll-like receptor ligands. M1 macrophages elevate their secretion of reactive oxygen and nitrogen species (ROS and NOS) and proinflammatory cytokines, to eliminate pathogens and damaged tissues while recruiting additional immune effector

cells. When most of the pathogens or tissue debris are removed, hyperactivation of macrophage bactericidal activity may result in unnecessary destruction of healthy tissue. As highly plastic cells, macrophages then respond to microenvironmental cues (e.g., T<sub>H</sub>2-type cytokines IL-4 and IL-13) and adopt an “alternatively activated” (M2) phenotype, which suppresses host defenses and facilitates wound healing and tissue remodeling to resolve inflammation and restore homeostasis at the inflamed site (52–55). This oversimplified segregation of macrophage phenotypes was originally applied to *in vitro* systems and has been widely used to provide a conceptual framework for subsequent research. However, given the complexity of *in vivo* microenvironments, macrophages exhibit phenotypes across a broad spectrum of activation states, and the simple M1/M2 dichotomy is unlikely to reflect physiological macrophage phenotypes (56–58).

Macrophages are crucial components in the pathogenesis of many inflammatory diseases, including atherosclerosis (59–61), IBDs (62–64), RA (65–68), and airway inflammation/asthma (49, 69–72). Lipid-laden macrophages are typically observed at atherosclerotic plaques. These maladaptive macrophages can induce a nonresolving inflammatory response leading to robust accumulation of cells, lipid, and matrix at the plaque. Defective macrophage efferocytosis (engulfment of dead cells) and enhanced apoptosis contribute to formation of a necrotic plaque core that might eventually rupture, causing platelet aggregation and thrombus formation (59, 60). The functional importance of myeloid cells in atherosclerosis is supported by experimental evidence that interventions to alter monocyte recruitment and/or survival can markedly affect disease progression (73–75). Airway inflammation typically accompanies airway allergic asthma, another disease involving macrophages. The microenvironment in asthma is dominated by type 2-associated cytokines (e.g., IL-4 and IL-13), which preferentially polarize macrophages into the M2 state (49). Elevated numbers of IL-4R<sup>+</sup> macrophages have been reported in asthmatic patients with defective lung function (76). Moreover, the presence of IL-4R<sup>+</sup> macrophages exacerbates allergen-induced airway inflammation, whereas reduction of IL-4R<sup>+</sup> macrophages alleviates this disease (77, 78).

Neutrophils, another major component of the innate immune response, are among the first cells recruited to inflammatory sites. These cells possess multiple means of eliminating invading pathogens, i.e., phagocytosis of microorganisms, degranulation to release antibacterial proteins, and emanation of neutrophil extracellular traps (NETs) (79, 80). Recently, many properties of neutrophils favoring the resolution of inflammation have been revealed (81), including production of annexin A1 (82) and lipid (e.g., LXA4 and 13-series resolvins) proresolution mediators (83, 84), chemokine/cytokine scavenging (e.g., CCL3 and CCL5) (85), and apoptosis-induced macrophage efferocytosis (86, 87). Similarly to macrophages, neutrophils are associated with multiple inflammatory syndromes, such as RA (88–90), chronic obstructive pulmonary disease (91, 92), and IBDs (51, 93). In IBDs, for example, neutrophils contribute to elimination of pathogens and immune cell (e.g., macrophages) recruitment and activation, as well as mucosal wound healing and resolution of inflammation. Of note, precise roles of neutrophils during intestinal inflammation are currently under investigation and are highly debated (51,



**Figure 1. Overview of the roles of HIF-1 $\alpha$  and HIF-2 $\alpha$  in myeloid cells.**

Both HIF-1 $\alpha$  and HIF-2 $\alpha$  are required for key macrophage functions, such as cytokine production and the ability to migrate and invade. However, macrophage glycolysis, ATP generation, and bactericidal activity have been related to HIF-1 $\alpha$  exclusively. Nevertheless, both isoforms contribute to pathogenesis of various acute inflammatory syndromes. Additionally, the roles of myeloid HIF- $\alpha$ s in the setting of tumor inflammation are currently being investigated. As compared with macrophages, less is known about HIF- $\alpha$ s in neutrophils. However, it is very clear that both isoforms are required to inhibit neutrophil apoptosis and elongate their lifespan. While HIF-1 $\alpha$  facilitates bacterial killing by neutrophils, many neutrophil functions seem less dependent on HIF-2 $\alpha$ , including respiratory burst, chemotaxis, and phagocytosis. Nevertheless, increased neutrophil HIF-2 $\alpha$  accumulation correlates with increased neutrophilic inflammation and lung injury in an LPS-induced acute lung injury murine model.

94). Some studies using colitis models, either chemically induced (dextran sulfate sodium [DSS] or dinitrobenzene sulfonic acid/trinitrobenzene sulfonic acid [DNBS/TNBS]) or immune system dysregulation-induced (CD4<sup>+</sup>CD45RB<sup>hi</sup> T cell transfer), showed that neutrophil depletion exacerbates colitis, suggesting a beneficial role of neutrophils in this setting (95, 96); however, other studies showed a completely opposite phenotype in which neutrophil depletion ameliorates colitis (97, 98). Additionally, the role of neutrophils could also depend on the concomitant presence of monocytes and macrophages (99). Therefore, while neutrophils are clearly associated with intestinal inflammation, whether they exert beneficial or detrimental effects appears to be model dependent and condition dependent.

## HIFs in myeloid cells

It is noteworthy that myeloid cells localize predominantly within hypoxic subdomains of tumors and sites of inflammation, and multiple mechanisms have been proposed to explain how hypoxia promotes recruitment and retention of myeloid cells (100). Both HIF-1 $\alpha$  and HIF-2 $\alpha$  regulate myeloid migratory activity: HIF-1 $\alpha$  is recruited to the *CXCR4* promoter, stimulating *CXCR4* transcription in human monocytes experiencing hypoxia (101). Moreover, *CXCR4* is a key chemokine receptor mediating chemotactic responses to CXCL12 ligand, which is upregulated in ischemic tissues such as arthritic joints (102). The HIF-1 $\alpha$ /PDK1 axis has been recently shown to contribute to macrophage migratory activity via induction of active glycolysis (103). For HIF-2 $\alpha$ , Casazza and colleagues demonstrated a semaphorin 3A/neuropilin1-dependent (SEMA3A/NRP1-dependent) means of macrophage positioning within the tumor. Here, NRP1 repression, which triggers macrophage retention in hypoxic regions, is mediated by HIF-2 $\alpha$ -dependent NF- $\kappa$ B activity (104).

Given the preferential localization of myeloid cells in hypoxic regions, significant efforts have defined how HIF-1 $\alpha$  and/or HIF-2 $\alpha$  promote myeloid cell adaptation to hypoxic environments and mediate inflammation (Figure 1). Cramer and colleagues were the

first to demonstrate the importance of HIF-1 $\alpha$  in macrophage and neutrophil function in the setting of inflammation (105). HIF-1 $\alpha$  was ablated in myeloid cells using lysozyme M (*LysM*) promoter-driven Cre recombinase, which is specific for the myeloid lineages, i.e., monocytes, macrophages, neutrophils, etc. Myeloid-specific *Hif1a* deletion results in defective glycolysis and ATP generation, leading to impairment of myeloid cell motility, invasiveness, aggregation, and bacterial killing. Moreover, mice with myeloid-specific HIF-1 $\alpha$  deficiency are protected against acute and chronic cutaneous inflammation and arthritis. Subsequent studies investigating HIF-1 $\alpha$  specifically in neutrophils demonstrated that hypoxia-induced inhibition of neutrophil apoptosis is dependent on HIF-1 $\alpha$  (106), and that HIF-1 $\alpha$  is required for phagocytes to fully exert their bactericidal activity (107). More recent attention has focused on myeloid cell immunometabolism (108, 109). Myeloid cells can undergo metabolic reprogramming to adapt to critical changes in the microenvironment. Tannahill and colleagues demonstrated that LPS exposure can alter glutamine-dependent anaplerosis (replenishment of TCA cycle intermediates) to elevate succinate levels, which further stabilize HIF-1 $\alpha$  in macrophages, resulting in increased production of IL-1 $\beta$  (110). These findings serve as an excellent example of how HIF-1 $\alpha$ -dependent immunometabolism can directly affect cytokine production by macrophages.

The role of HIF-2 $\alpha$  in myeloid cells has also been investigated using the *LysM-Cre*-mediated deletion strategy. Macrophages lacking HIF-2 $\alpha$  exhibit defects in the production of inflammatory cytokines/chemokines in response to hypoxia, migration, and invasion. Myeloid HIF-2 $\alpha$  deficiency also protects mice in models of sepsis, cutaneous inflammation, peritonitis, hepatocellular carcinoma, and colitis-associated colorectal cancer (CAC) (111). Like HIF-1 $\alpha$ , neutrophil HIF-2 $\alpha$  contributes to hypoxia-induced inhibition of apoptosis. HIF-2 $\alpha$  deficiency increases neutrophil apoptosis in vivo and ex vivo, leading to suppression of neutrophilic inflammation and inflammatory responses during acute lung injury (112).

One interesting observation from the work of Imtiyaz and colleagues (111) is that, unlike myeloid HIF-1 $\alpha$ , HIF-2 $\alpha$  deficiency

Table 1. Summary of myeloid HIF's role in various inflammatory scenarios

Inflammatory disease	HIF subunit	Overall effect	Proposed mechanisms	Reference
Atherosclerosis	HIF-1α	Promotes inflammation	Enhances lipid uptake Induces sterol synthesis Suppresses cholesterol efflux Elevates proteoglycan secretion Promotes angiogenesis Increases glycolytic flux Sustains viability Upregulates proinflammatory cytokine gene expression	118–122, 124
	HIF-2α	Promotes inflammation	Elevates proteoglycan secretion	122
Adipose tissue inflammation/obesity	HIF-1α	Promotes inflammation	Enhances macrophage M1 polarization	138
	HIF-2α	Suppresses inflammation	Represses NO and proinflammatory cytokines production from macrophages Improves insulin resistance in adipocytes	139
		No effect		46
Sepsis	HIF-1α	Promotes inflammation	Proinflammatory cytokine production	39, 144
	HIF-2α	Promotes inflammation	Maintains serum levels of proinflammatory cytokines Lowers IL-10 level	111
Airway allergy and asthma	HIF-1α	Promotes inflammation	Promotes VEGF and CXCL1 expression Enhances eosinophil infiltration	149, 150
		Suppresses inflammation	Elevates IL-10 level to block dendritic cell and T helper cell response Inhibits neutrophil apoptosis	147, 148
Gastritis	HIF-1α	Suppresses inflammation	?	153
Renal fibrosis and inflammation	HIF-1α	Suppresses inflammation	Represses CCR2 and CCL2 expression to inhibit macrophage infiltration Suppresses CTGF production from renal cells	157, 158
	HIF-2α	Suppresses inflammation	Represses CCR2 and CCL2 expression to inhibit macrophage infiltration	157
Arthritis	HIF-1α	Promotes inflammation	?	105
Cutaneous inflammation	HIF-1α	Promotes inflammation	?	105
	HIF-2α	Promotes inflammation	Increases neutrophil infiltration	111

cy does not alter cellular ATP production. The notion that HIF-1α and HIF-2α exert nonredundant or even opposing functions in macrophages is further supported by a study showing that HIF-1α and HIF-2α differentially regulate NO production by controlling expression of iNOS and arginase 1, respectively (113). Given the complex roles of HIF-1α and HIF-2α in macrophages, pan-HIF inhibition via pharmacological or genetic methods (i.e., *Arnt* deletion) is warranted. In a murine CAC model, treatment with the HIF inhibitor acriflavine reduces both tumor burden and macrophage infiltration (114). Additionally, myeloid cell-specific ARNT deficiency reduces macrophage proinflammatory cytokine production, and mice lacking myeloid ARNT are protected from cutaneous inflammation and exhibit delayed wound healing (115).

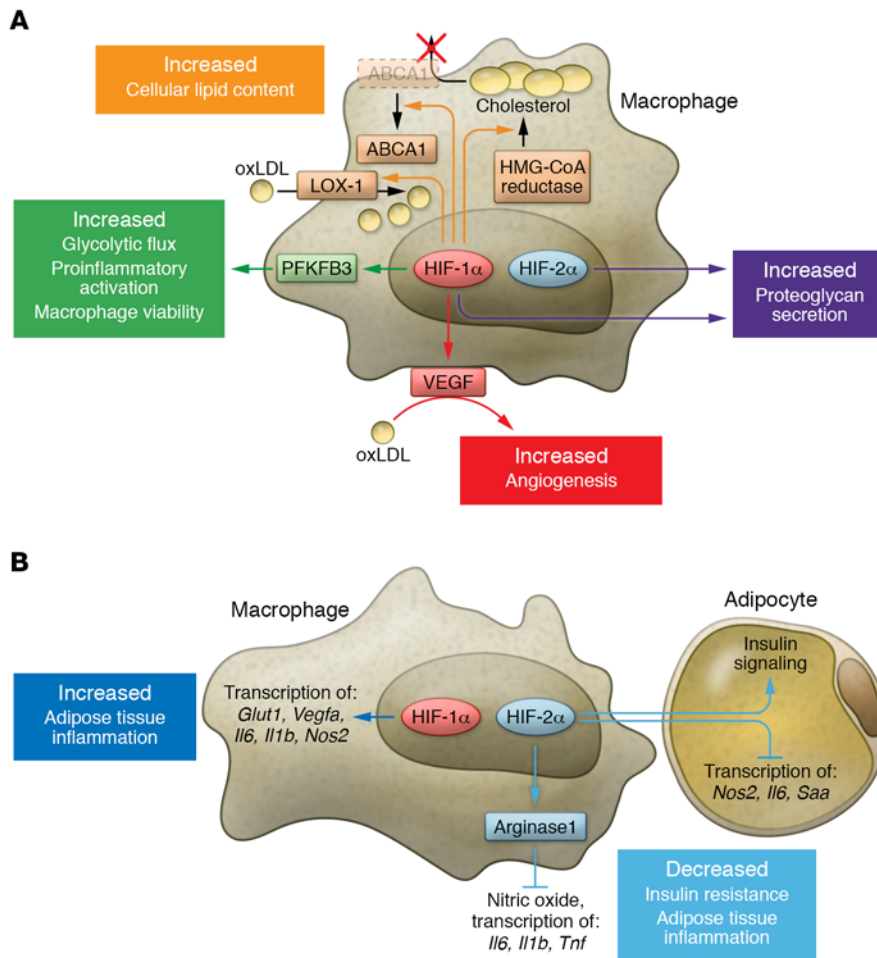
In the following sections, we will discuss the roles of myeloid HIF-αs in the settings of specific inflammatory diseases, as summarized in Table 1.

**Atherosclerosis.** Atherosclerosis is a chronic inflammatory disease of the arterial vasculature. Retention of apolipoprotein B-containing lipoproteins and accumulation of cholesterol-laden macrophages in the artery wall contribute to this syndrome. Monocytes are first recruited to differentiate into mononucle-

ar phagocytes and ingest lipoproteins; however, lipid buildup in these cells transforms them into foam cells that exhibit dysregulated lipid metabolism and elevated secretion of proinflammatory cytokines (e.g., IL-6 and TNF-α) and macrophage retention factors (i.e., netrin 1 and semaphorin 3E). Foam cells promote the further progression of atherosclerosis (59, 60).

Elevated levels of HIF-1α and HIF-2α are detected in human atherosclerotic carotid plaques compared with normal arteries, where HIF-1α colocalizes with CD68, a macrophage marker (116). Hypoxia has been implicated as a pathogenic factor in atherosclerosis and contributes to the proatherosclerotic functions of macrophages (Figure 2A) (117). Several reports showed that lipid uptake and foam cell formation are dependent on hypoxia and HIF-1α (118–120). Both murine and human macrophage cell lines increase cellular neutral lipid content when cultured under hypoxic conditions; however, this effect is reversed upon HIF-1α depletion. Multiple HIF-1α-dependent mechanisms have been proposed for this phenotype. For example, hypoxia enhances expression of lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1), which promotes oxLDL uptake in the murine macrophage cell line RAW264.7; silencing of HIF-1α diminishes the





**Figure 2. Context-dependent myeloid HIF- $\alpha$  effector functions.** Myeloid HIF-1 $\alpha$  and HIF-2 $\alpha$  exhibit diverse functions that differ in distinct pathological settings. The two isoforms sometimes work in a similar fashion, but can also oppose each other. **(A)** In the setting of atherosclerosis, both myeloid HIF-1 $\alpha$  and HIF-2 $\alpha$  contribute to pathogenesis. HIF-1 $\alpha$  promotes lipid uptake in macrophages through induction of LOX-1. Elevation in HMG-CoA reductase activity and surface ABCA1 perinuclear relocation downstream of HIF-1 $\alpha$  increases cholesterol synthesis while simultaneously blocking cholesterol efflux. Through VEGF production, myeloid HIF-1 $\alpha$  also facilitates oxLDL's proangiogenic effects. Regulation of PFKFB3 by HIF-1 $\alpha$  enhances glycolytic flux and is crucial for both viability and proinflammatory activation of macrophages. Both isoforms contribute to proteoglycan secretion. **(B)** In adipose tissue of obese subjects, ATM HIF-1 $\alpha$  enhances inflammation via induction of hypoxic and proinflammatory genes, while ATM HIF-2 $\alpha$  alleviates insulin resistance and adipose tissue inflammation. ATM HIF-2 $\alpha$  not only suppresses proinflammatory responses in ATM via induction of arginase 1 (ARG1) expression, but also sensitizes adipocytes to insulin signaling while inhibiting proinflammatory gene transcription. Abbreviations: oxLDL, oxidized low-density lipoprotein; LOX-1, lectin-like oxLDL receptor-1; ABCA1, ATP-binding cassette subfamily A member 1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; *Glut1*, glucose transporter 1; *Saa*, serum amyloid A.

upregulation of LOX-1 (119). Hypoxic J774 murine macrophages exhibit elevated sterol accumulation due to (a) enhanced sterol synthesis via increased 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity and (b) suppressed cholesterol efflux due to altered subcellular localization of ATP-binding cassette subfamily A member 1 (ABCA1) (120). These phenotypic changes are also HIF-1 $\alpha$  dependent. In U937 human monocytes, oxLDL treatment increases the expression of 70 out of 96 key genes that are known to be involved in atherosclerosis, while 57 of these genes (e.g., cyclooxygenase-2 [COX-2], vascular cell adhesion molecule [VCAM-1], and IL-1 $\beta$ ) are downregulated with HIF-1 $\alpha$  siRNA pretreatment (118). Other proatherosclerotic functions of macrophages, such as promotion of angiogenesis and proteoglycan synthesis, are also dependent on HIF- $\alpha$ s (121, 122). In a coculture system of human monocytes/macrophages and endothelial cells, oxLDL strongly induces *HIF1A* and *VEGFA* expression in macrophages, while increasing endothelial cell tube formation. Of note, oxLDL proangiogenic effects are partially lost upon HIF-1 $\alpha$  inhibition (121). The notion that myeloid HIF-1 $\alpha$  can promote angiogenesis through VEGF upregulation is demonstrated in other studies as well (123). Macrophages can also contribute to pathogenesis by secreting proteoglycans such as versican, which modulate lipoprotein retention and the activity of enzymes, cytokines, and other growth factors in atherosclerotic lesions. Increased versican and perlecan expression is detected in macrophages under hypoxia;

versican is coregulated by HIF-1 $\alpha$  and HIF-2 $\alpha$ , while perlecan is only dependent on HIF-1 $\alpha$  (122). Myeloid HIF-1 $\alpha$  is also a critical regulator of both glycolytic metabolism and proinflammatory activation of macrophages, and is stabilized by cues in the atherosclerotic microenvironment, such as hypoxia and cytokines. HIF-1 $\alpha$  increases transcription of the gene encoding 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), a key enzyme in the glycolytic pathway, leading to (a) increased glycolytic flux, (b) increased proinflammatory cytokine production (e.g., TNF- $\alpha$ ), and (c) maintenance of macrophage viability (124). Together, these studies reveal that HIF- $\alpha$ s are crucial components in determining macrophage proatherosclerotic functions.

Unfortunately, in vivo studies do not always provide consistent findings. In a wire-induced vascular injury model, myeloid HIF-1 $\alpha$  promotes vascular inflammation and remodeling manifested by increases in TNF- $\alpha$  and IL-6 levels proximal to the injury site and neointimal thickening of injured arteries (125). However, a recent in vivo genetic and drug-based approach suggested the opposite effect in a different mouse model, indicating that HIF-1 $\alpha$  and HIF-2 $\alpha$  accumulation correlates with reduced atherosclerosis development. The authors inhibited PHD2, resulting in HIF-1 $\alpha$  and HIF-2 $\alpha$  stabilization, by administering a pharmacological inhibitor (FG-4497) in an LDL receptor-deficient model of atherosclerosis or by crossing *Hif-p4h-2* hypomorphic (*Hif-p4h-2<sup>gt/gt</sup>*) mice with LDL receptor-deficient mice. PHD2 inhibition led to

reductions in levels of atherosclerotic plaque formation, weight gain, insulin resistance, liver and white adipose tissue (WAT) mass, adipocyte size, number of inflammation-associated WAT macrophage aggregates, and high-fat diet-induced increases in serum cholesterol levels (126). The discrepancy with previous findings could be due to non-myeloid-specific inhibition of PHD2 *in vivo*. As such, the *in vivo* role of myeloid HIF signaling in atherosclerosis requires further investigation.

**Adipose tissue inflammation and obesity.** Adipose tissue hypoxia, chronic inflammation, and macrophage infiltration are key characteristics of obesity (127–130). In lean mice, a majority of the adipose tissue macrophages (ATMs) are alternatively activated M2 macrophages, which suppress proinflammatory responses and maintain adipocyte insulin sensitivity by elevated expression of arginase 1 and IL-10, among other factors. In the setting of obesity, the number of macrophages is increased and their phenotype altered. Many macrophages are in a classically activated (M1) state, which produces NO and secretes proinflammatory cytokines, such as IL-1 $\beta$ , TNF- $\alpha$ , and IL-6. These cytokines potentiate inflammatory responses in adipose tissue that eventually result in insulin resistance (129, 131–133). However, a shift in macrophage polarization in lean versus obese humans is debated. Aron-Wisnewsky and colleagues reported a more M1 than M2 polarization of macrophages (defined by CD40 and CD206 expression, respectively) in obese patients, which shifts to a less proinflammatory profile after weight loss (134). A more recent study, however, demonstrated that even though macrophage numbers increase in adipose tissue of obese patients, most of these ATMs are predominantly M2 macrophages (defined by CD163 and IL-10 expression) (135). These contrasting observations may be a consequence of the oversimplified dichotomy of macrophage polarization (see above). In the work of Wentworth and colleagues, ATMs were found to be positive for both M1 (CD11c) and M2 (CD206) markers, exhibiting a proinflammatory status associated with insulin resistance in obese humans (136).

In obese patients, higher HIF-1 $\alpha$  levels are evident in adipose tissue (137), and hypoxia and HIF signaling regulate ATM functions in the setting of obesity (Figure 2B). Fujisaka and colleagues showed that adipose tissue hypoxia induces proinflammatory phenotypes of M1 ATMs, with elevated expression levels of proinflammatory cytokines and hypoxia-related genes (138). In contrast with HIF-1 $\alpha$ 's proinflammatory roles in ATMs, macrophage HIF-2 $\alpha$  has been suggested to ameliorate adipose tissue inflammation and insulin resistance (139). Choe and colleagues demonstrated that HIF-2 $\alpha$  overexpression in macrophages represses NO production and expression of proinflammatory cytokine genes. On the other hand, silencing HIF-2 $\alpha$  in palmitate-treated macrophages increases NO production, indicating that HIF-2 $\alpha$  is required to downregulate palmitate-induced NO production. Macrophage HIF-2 $\alpha$  also regulates the crosstalk between macrophages and adipocytes. Adipocytes cocultured with wild-type macrophages exhibit decreased insulin signaling, while coculture with HIF-2 $\alpha$ -deficient macrophages not only reverses the decrease in insulin signaling, but also stimulates adipocyte proinflammatory responses. In a murine model of high-fat diet-induced obesity, HIF-2 $\alpha$  haplo-deficient (*Epas1*<sup>+/-</sup>, “*Hif2a*<sup>+/-</sup>” herein) mice were more susceptible to adipose tissue inflammation and became insulin resistant.

Upon macrophage depletion, both insulin resistance and adipose tissue inflammation improved in this model (139). In summary, myeloid HIF-1 $\alpha$  promotes adipose tissue inflammation by aiding macrophage M1 polarization, while myeloid HIF-2 $\alpha$  constrains the inflammatory response and insulin resistance in adipose tissue. These conclusions are consistent with the understanding that different polarization states of macrophages exert opposite effects on adipose tissue inflammation, and are also consistent with the notion that HIF-1 $\alpha$  is required for M1 polarization of macrophages, and HIF-2 $\alpha$  for M2 polarization (113). However, another study suggests that hypoxia potentiates palmitate-induced expression of the proinflammatory genes IL-6 and IL-1 $\beta$  independently of HIF-1 $\alpha$  and HIF-2 $\alpha$  in human macrophages. Instead, their induction occurs via activation of JNK and p38 MAPK signaling (46). Another group proposed that insulin resistance and metabolic dysregulation in obese mice are mainly regulated by adipocyte HIF-2 $\alpha$ , but not myeloid HIF-2 $\alpha$  (140). Clearly, additional effort is needed to determine the extent of HIF- $\alpha$ -mediated regulation of ATM phenotypes and whether HIF- $\alpha$ -dependent ATM phenotypic changes are sufficient to alter adipose tissue inflammation and obesity.

**Sepsis.** Sepsis is a life-threatening systemic illness that is normally induced by microbial infection and may result in fatal multi-organ failure in patients. Hyperactivation of the innate immune system is believed to be a key component of this pathophysiology. Macrophages and neutrophils release cytokines, chemokines, and complement-activation mediators soon after the initial microbial stimuli (141–143). LPS, a lipoglycan found in the outer membrane of gram-negative bacteria and often used to induce murine sepsis or endotoxemia, has been shown to stabilize macrophage HIF-1 $\alpha$  via p42/44 MAPK and NF- $\kappa$ B signaling pathways (40). HIF-1 $\alpha$  subsequently promotes macrophage *in vitro* production of proinflammatory cytokines such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IL-1 $\alpha$ , IL-4, and IL-12. When mice with conditional *Hif1a* deletion in the myeloid lineage are challenged with LPS, they exhibit reduced hypothermia and hypotension, along with enhanced survival compared with mice that express myeloid HIF-1 $\alpha$  (39). Myeloid HIF-1 $\alpha$  deficiency is also protective in a gram-positive endotoxin-induced murine sepsis model (144). Similar to HIF-1 $\alpha$ , deletion of myeloid HIF-2 $\alpha$  is also protective against sepsis. Cultured bone marrow-derived macrophages (BMDMs) isolated from mice with myeloid HIF-2 $\alpha$  deficiency also exhibit decreased proinflammatory cytokine and increased antiinflammatory cytokine production in response to LPS stimulation. Additionally, myeloid-specific HIF-2 $\alpha$  deficiency promotes survival in LPS-challenged mice (111). Collectively, these data show that both HIF-1 $\alpha$  and HIF-2 $\alpha$  contribute to macrophages' pathogenic roles in septic pathology. This conclusion is further supported by a more recent study in which 2-methoxyestradiol (2-ME2), a HIF-1 $\alpha$  inhibitor (145), protected mice from both LPS- and cecal ligation and puncture-induced (CLP-induced) sepsis. Suppression of cytokines by 2-ME2 was observed in LPS-stimulated peritoneal macrophages, indicating that macrophage phenotypic alterations also contributed to the survival phenotype (146).

**Airway allergy and asthma.** Although airway allergy is a chronic inflammatory disease primarily driven by DCs and Th2 T lymphocytes, lung macrophages have also been implicated in airway inflammation and asthma (69–71). In a house dust mite (HDM) antigen-induced experimental model of airway allergy, myeloid

HIF-1 $\alpha$  deficiency renders mice more susceptible to these stimuli (147). Toussaint and colleagues found that lung macrophage HIF-1 $\alpha$  drives expression of immunosuppressive IL-10 to impair DC activation and Th responses (147). A similar protective effect of myeloid HIF-1 $\alpha$  is also evident in the setting of pulmonary fungal infections. Shepardson and colleagues found that mice with myeloid HIF-1 $\alpha$  deficiency are more susceptible to pulmonary challenge with *Aspergillus fumigatus*, are defective in fungal clearance, and exhibit decreased lung neutrophil numbers. These phenotypes can be partly attributed to decreased production of CXCL1 and increased neutrophil apoptosis (148). Contradictory to the finding that macrophage HIF-1 $\alpha$  prevents airway allergy, a study by Byrne and colleagues suggests that development of airway allergy is dependent on macrophage HIF-1 $\alpha$ . They demonstrated that HDM increases HIF-1 $\alpha$  abundance in the lung, inducing VEGF and CXCL1 production in primary lung macrophages in a HIF-1 $\alpha$ -dependent manner. Pharmacological HIF-1 $\alpha$  inhibition in this model suppresses pulmonary allergic inflammation and VEGF and CXCL1 secretion (149). Using an ovalbumin-induced (OVA-induced) asthma model, others have shown that myeloid HIF-1 $\alpha$  deficiency reduces airway hyperresponsiveness and eosinophil infiltration. Furthermore, HIF-1 $\alpha$  and HIF-2 $\alpha$  directly regulate eosinophil chemotaxis in opposing ways (150). Therefore, the role of myeloid HIF-1 $\alpha$  in airway diseases remains very complex, and varies in different experimental models.

**Gastritis.** Inflammation in the gastric mucosa is most commonly induced by *Helicobacter pylori* infection in humans. Chronic gastritis may progress to gastrointestinal ulcers or gastric cancer (151). Like other inflammatory diseases, recruitment of immune cells is also evident during gastric inflammation, and macrophage depletion using drug-loaded liposomes has been shown to ameliorate the pathology of *H. pylori*-induced gastritis (152). A recent report (153) specifically examined the role of myeloid HIF-1 $\alpha$  in gastritis. The authors found that HIF-1 $\alpha$  levels are positively correlated with the severity of gastritis in patients with *H. pylori* infections, and HIF-1 $\alpha$  is readily observed in macrophages from patient biopsies. In vitro, *H. pylori* preferentially upregulates *Hif1a* and downregulates *Hif2a* transcription in BMDMs, while expression of proinflammatory cytokines is dependent on HIF-1 $\alpha$ . Elevated HIF-1 $\alpha$  levels also contribute to bactericidal activity of both neutrophils and macrophages. Interestingly, in a murine model of *H. pylori*-induced gastritis, mice with myeloid-specific HIF-1 $\alpha$  deletion failed to exhibit changes in bacterial loads as compared with wild-type animals. Even though myeloid-specific HIF-1 $\alpha$  deficiency blocks the induction of proinflammatory gene expression upon *H. pylori* infection, more severe gastritis is observed in these animals, characterized by worsened histopathological grading, greater immune cell infiltration, and a higher cellular proliferation index compared with infected wild-type animals (153). Overall, myeloid HIF-1 $\alpha$  appears to be protective in *H. pylori*-mediated gastritis; however, additional work is needed to fully explain these counterintuitive phenotypes.

**Renal fibrosis and inflammation.** Macrophages represent the dominant infiltrating cell type during progression of chronic kidney disease (CKD), driven partially by low O<sub>2</sub> availability in the kidney (154–156). Kobayashi and colleagues addressed the role of myeloid HIF- $\alpha$ s using the typical *LysM-Cre* strategy in a murine

unilateral ureteral obstruction-induced (UUO-induced) kidney injury model. Activation of myeloid HIF via *LysM-Cre*-driven *Vhl* deletion attenuates renal inflammation, while deletion of both myeloid *Hif1a* and *Hif2a* enhances inflammation, as indicated by increased F4/80<sup>+</sup> cell numbers in the kidney. However, the presence of myeloid HIF- $\alpha$ s does not alter renal fibrosis. The authors suggest that hypoxia and/or myeloid HIF- $\alpha$  activation alleviates renal inflammation via suppression of *Ccr2* and *Ccl2*, which are crucial for monocyte recruitment (157). The notion that myeloid HIF-1 $\alpha$  regulates UUO-induced nephropathy is further supported by another study using the same *LysM-Cre* model; however, Tateishi and colleagues reported that myeloid HIF-1 $\alpha$  deletion promoted renal fibrosis but did not alter macrophage accumulation in the UUO model. They suggested a different mechanism for the protective role of myeloid HIF-1 $\alpha$  in renal fibrosis: suppression of renal connective tissue growth factor (CTGF) within renal cells (158). The discrepancy between the two reports could be due to deletion of two isoforms of HIF- $\alpha$  versus deletion of HIF-1 $\alpha$  alone. Nevertheless, both studies suggest a protective role for myeloid HIF- $\alpha$ s in CKD, which partly supports the observation in patients with CKD that elevated renal HIF-1 $\alpha$  expression correlates with less severe disease (159).

**Cancer-associated inflammation.** A strong link between chronic inflammation and tumor progression has been clearly evident for some time. For example, patients with IBDs are at increased risk of developing colorectal cancer (160–162). Similar to sites of inflammation, the tumor microenvironment is also highly hypoxic. Macrophages predominantly accumulate in hypoxic regions, change their gene expression profiles in response to low O<sub>2</sub>, and function in response to limited O<sub>2</sub> availability (100). Significant effort has delineated the respective roles of myeloid HIF-1 $\alpha$  and HIF-2 $\alpha$  in the tumor setting, beyond the two examples we will summarize here. In a PyMT model of breast cancer, loss of myeloid HIF-1 $\alpha$  significantly decreases tumor mass and inhibits tumor progression, likely through suppression of cytotoxic T cell response to the tumors (163). As for HIF-2 $\alpha$ , Imtiyaz and colleagues demonstrated that myeloid HIF-2 $\alpha$  deficiency leads to reduced tumor burden and progression in a murine CAC model, while ablating macrophage infiltration of murine hepatocellular carcinoma. The authors suggest that these results could partly be due to defective migration and invasion of macrophages with HIF-2 $\alpha$  loss (111). For a more comprehensive discussion of myeloid HIF- $\alpha$ s in cancer, please refer to other reviews within this series (164, 165).

## Summary

Overall, hypoxia and inflammation are clearly inextricably linked. Hypoxia can be a strong contributory factor in certain inflammatory diseases; in turn, inflammation sites often exhibit low O<sub>2</sub> tension. Myeloid cells are major components of innate immunity that are tightly associated with inflammation in different tissues and found predominantly localized within the hypoxic regions of inflamed tissues. Myeloid cell infiltration on its own can contribute to O<sub>2</sub> deprivation at these sites. In response to hypoxia, myeloid cells stabilize HIF- $\alpha$ s, which facilitates their metabolic reprogramming and other adaptations, allowing myeloid cells to take on transient roles in different stages of disease progression. In many types of inflammation described in this review,



the roles of myeloid HIF- $\alpha$ s remain incompletely described. In many cases, myeloid HIF-1 $\alpha$  and HIF-2 $\alpha$  have nonredundant or even opposing effects on myeloid cell functions (20). Therefore, many questions concerning the role of HIF- $\alpha$ s in myeloid cells require further investigation. For example, in specific inflammatory diseases, it is unclear if it would be beneficial or detrimental to target HIF- $\alpha$ s. If targeting of HIF- $\alpha$ s is beneficial, then should a specific HIF- $\alpha$  isoform or both isoforms be targeted? Even if all mechanisms mediated by HIF in inflammatory myeloid cells are elucidated, clinical translation will still be challenging. For example, how can HIF be specifically targeted in myeloid cells? How efficient will these therapies be? Nevertheless, oxygen-sensing

pathways in myeloid cells are clearly key determinants of their physiological and pathological functions and these pathways remain attractive therapeutic targets.

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