

# HIF1 $\alpha$ and metabolic reprogramming in inflammation

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**HIF1 $\alpha$  is a common component of pathways involved in the control of cellular metabolism and has a role in regulating immune cell effector functions. Additionally, HIF1 $\alpha$  is critical for the maturation of dendritic cells and for the activation of T cells. HIF1 $\alpha$  is induced in LPS-activated macrophages, where it is critically involved in glycolysis and the induction of proinflammatory genes, notably *Il1b*. The mechanism of LPS-stimulated HIF1 $\alpha$  induction involves succinate, which inhibits prolyl hydroxylases (PHDs). Pyruvate kinase M2 (PKM2) is also induced and interacts with and promotes the function of HIF1 $\alpha$ . In another critical inflammatory cell type, Th17 cells, HIF1 $\alpha$  acts via the retinoic acid-related orphan receptor- $\gamma$ t (ROR $\gamma$ t) to drive Th17 differentiation. HIF1 $\alpha$  is therefore a key reprogrammer of metabolism in inflammatory cells that promotes inflammatory gene expression.**

## Introduction

ATP production is required to support physiological function in all cells. Methods of ATP production vary between cell types and cellular activation states. Glucose can be used to fuel ATP production through two linked metabolic pathways: glycolysis and the TCA cycle. In glycolysis, glucose is converted into pyruvate in the cytoplasm and phosphates are transferred to ADP to generate two molecules of ATP. Pyruvate can also be converted into acetyl-CoA, which enters the TCA cycle, linking the two processes. The TCA cycle produces NADH and flavin adenine dinucleotide (FADH<sub>2</sub>), which are used to fuel oxidative phosphorylation (OXPHOS) in the mitochondria to produce an additional 36 molecules of ATP. Cells can utilize other substrates aside from glucose depending on the context. Fatty acids and glutamine can both be used to fuel OXPHOS in some cells (1, 2).

A key feature of metabolic pathways is their plasticity. Changes in nutrient availability or oxygen levels are the best-characterized drivers of metabolic reprogramming. For example, hypoxia is a well-known driver of glycolysis, as an oxygen deficit results in limited OXPHOS. Under these circumstances cells must rely on glycolysis to generate ATP. HIF1 $\alpha$  is critical for this process, as it induces the expression of glycolytic enzymes such as hexokinase and phosphofructokinase, thereby allowing for sustained ATP production (3, 4). Hypoxia and inflammation are inherently linked. Decreasing oxygen levels induce metabolic changes to sustain ATP production. Similarly, quiescent immune cells can be viewed as metabolically inert and require significant metabolic reprogramming upon activation to provide sufficient ATP for effector functions. The HIF pathway provides a switch through which metabolic phenotypes can be amended in both of these scenarios and therefore is a critical transcriptional regulator of immunity and inflammation (2).

HIF is a highly conserved member of the PER-ARNT-SIM (PAS) subfamily of the basic helix-loop-helix (bHLH) family of

transcription factors (5). During active HIF signaling, HIF forms a heterodimeric complex that consists of an  $\alpha$  and a  $\beta$  subunit. The  $\alpha$  subunit can be of two primary forms, HIF1 $\alpha$  or HIF2 $\alpha$ . The  $\beta$  subunit is the constitutively expressed aryl hydrocarbon receptor nuclear translocator (ARNT). Upon dimerization, the HIF $\alpha$ /ARNT complex translocates to the nucleus where it binds to the promoters of target genes containing hypoxia response elements (HREs). This binding initiates transcription of a battery of genes involved in cellular adaptation to hypoxia, metabolism, and cell function. HIF signaling is primarily regulated by the stability of its  $\alpha$  subunit. In a resting cell, HIF1 $\alpha$  is hydroxylated at conserved proline residues by the prolyl hydroxylases (PHDs). This hydroxylation allows for HIF1 $\alpha$  ubiquitination by the von Hippel-Lindau (VHL) E3 ubiquitin ligase, marking it for rapid proteasomal degradation. The PHDs are oxygen dependent; thus, under normoxic conditions, HIF1 $\alpha$  is continuously turned over by means of degradation, resulting in low basal HIF1 $\alpha$  levels. In normoxia HIF1 $\alpha$  has a remarkably short half-life of less than 5 minutes (6). Hypoxic conditions result in PHD inhibition and an attenuation of HIF1 $\alpha$  hydroxylation. In the absence of proteasomal degradation, HIF1 $\alpha$  accumulates, translocates to the nucleus, and increases transcription of HRE-containing genes.

In addition to changing levels of oxygen, another well-characterized example of a HIF-mediated switch to glycolysis has been described in tumors, whereby under normoxia, glycolysis still predominates. This metabolic switch has been termed the Warburg effect (7) and can be driven by mutations in proto-oncogenes such as *Myc* or *Ras*, which ultimately result in HIF1 $\alpha$ -mediated metabolic reprogramming towards a glycolytic phenotype. Hypoxia is a prominent component of solid tumors, primarily as a pathophysiological consequence of disturbed microcirculation due to insufficient vascularization following rapid tumor growth. HIF1 $\alpha$  plays an important role in modifying tumor metabolism in response to an increasingly inhospitable hypoxic microenvironment. The changes in gene expression induced by HIF signaling contribute to many of the hallmarks of cancer that enable tumor growth, survival, and invasion. Hypoxia is seen as a poor prognostic marker in many cancer types. Importantly, HIF-inducible

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effectors of the transcriptional response to tumor hypoxia include carbonic anhydrase IX (CAIX), monocarboxylate transporter 4 (MCT4), and programmed death-ligand 1 (PD-L1). CAIX plays a pivotal role in biological processes required for cancer progression and it is expressed in the hypoxic regions of many types of solid tumor (8). MCT4 is involved in the export of lactate from tumor cells and contributes to metastasis and angiogenesis (9, 10). PD-L1 expression is also upregulated by HIF signaling and its expression is correlated with decreased cytolytic action of T cells (11). To date, no selective HIF1 $\alpha$  inhibitor has been clinically approved, but it is clear that targeting key downstream effectors of hypoxia, or HIF1 $\alpha$  itself, is therapeutically advantageous in preclinical tumor models (12, 13).

The nutrient sensor mTORC1 induces metabolic reprogramming in a HIF-dependent manner. Activation of mTORC1 promotes a glycolytic phenotype through the upregulation of glycolytic genes such as the glucose transporter 1 (GLUT1). mTORC1 activation also promotes cholesterol and fatty acid synthesis through a pathway involving SREBPs and PPAR $\gamma$ . mTORC2 activation promotes metabolic reprogramming by activating MYC and AKT, which similarly induce transcription of the glycolytic apparatus (14, 15).

A metabolic switch also occurs following oncogenic mutations in succinate dehydrogenase (SDH), which leads to succinate accumulation. Succinate inhibits PHDs, stabilizing HIF1 $\alpha$  to create a state termed pseudohypoxia. The stabilization of HIF1 $\alpha$  induces increased expression of the glycolytic machinery (16). Importantly, this stabilization occurs independently of hypoxia.

As mentioned above, a HIF-mediated metabolic reprogramming event is also seen during the activation of certain immune cells. Aerobic glycolysis and HIF1 $\alpha$  signaling are key features of activated immune cells, the best example being in macrophages activated by the gram-negative bacterial product LPS (17), or in Th17 cells, which produce the proinflammatory cytokine IL-17 (18). These findings indicate that metabolic reprogramming drives specific functions in immune cells, attesting to the crucial role of HIF1 $\alpha$  in inflammation. This review will detail the role of HIF-mediated signaling in the metabolic reprogramming of macrophages, dendritic cells (DCs), T-cells, and neutrophils.

### HIF1 $\alpha$ and immune-related genes

HIF1 $\alpha$  is expressed in several types of innate immune cells, including macrophages, DCs, neutrophils, and Th17 cells (19–22). In these cells, HIF1 $\alpha$  plays a fundamental role in the response to pathological stress as well as environmental adaptation. Immune cells have varied energy requirements depending on their activation state and must be able to alter their metabolic profile accordingly. The HIF pathway provides these cells with a metabolic switch, allowing them to respond appropriately to the significant changes in energy requirements that occur upon activation, as well as adapt to the hypoxic conditions that might prevail in inflamed tissue. Foci of inflammation, generated by a plethora of conditions such as tissue insult, infection, or auto-immune responses, can represent a difficult microenvironment for a cell. Hypoxia, acidosis, redox stress, and hypoglycemia are common features that cells must adapt to in order to survive. The role of HIF in environmental adaptation marks it as a cru-

cial pathway in local and infiltrating innate immune cells under these suboptimal conditions.

While historically the HIF pathway has been implicated solely in this adaptation to the environmental milieu, multiple studies have detailed a clear role for HIF signaling that is independent of environmental changes in some cell subsets. The HIF pathway has been linked to the key metabolic changes that occur following innate immune cell activation downstream of pattern recognition receptor (PRR) ligation and other signaling events, which mirror some of the hallmark metabolic changes seen in cancer cells (23).

There is an important association between hypoxia and inflammation. A classic example of a hypoxic inflammatory setting is in joints affected by rheumatoid arthritis (RA), where areas of hypoxia and inflammation coincide. RA is characterized by chronic inflammation of the synovium of joint tissues, leading to localized erosion of these tissues and debilitating pain and deformity. The hypoxic RA joint is highly infiltrated with synovocytes, lymphocytes, and macrophages (24). Similarly, colitis is characterized by foci of coinciding inflammation and hypoxia (25). Mountain sickness exemplifies the correlation between oxygen tension and inflammation. Decreased blood oxygen has been linked with increased inflammatory cytokines circulating in blood; these hypoxia-induced cytokines are seen following exposure to high altitudes. Altitudes greater than 3,400 meters evoked an increase in circulating proinflammatory IL-6 in healthy volunteers (26, 27).

Hypoxia can increase mitochondrial ROS, which have downstream effects on HIF1 $\alpha$  stability. Increased mitochondrial ROS is promoted by the oxidation of cysteine residues in the double-stranded  $\beta$ -helix fold that constitutes the catalytic site of PHDs. The oxidative PHD2 dimerization that follows cysteine oxidation is associated with the stabilization and activation of HIF1 $\alpha$  under oxidative stress, and therefore promotes aerobic glycolysis (28). Mitochondrial ROS also stabilize HIF1 $\alpha$  by inducing decarboxylation of  $\alpha$ -ketoglutarate ( $\alpha$ -KG) (29) and by oxidizing iron (Fe<sup>2+</sup>) to Fe<sup>3+</sup>; both Fe<sup>3+</sup> and  $\alpha$ -KG are necessary PHD cofactors (30). ROS impair the activity of another key member of the 2-oxoglutarate-dependent dioxygenase family, factor inhibiting HIF (FIH) (30). Similarly to the PHD enzymes, FIH activity promotes HIF degradation. Antimycin-induced superoxide production was suppressed by small-molecule inhibitors, which concurrently inhibited HIF1 $\alpha$  stabilization without directly affecting metabolism (31). Cells that lack mitochondria (known as  $\rho^0$  cells) failed to induce HIF1 $\alpha$  accumulation in response to hypoxia (32); therefore, maintenance of mitochondrial membrane potential is essential not only for efficient bioenergetic function, but also for cell proliferation and oxygen sensing (33). The emergence of a role for mitochondria as signaling organelles for cellular responses has particular relevance in inflammation, where HIF1 $\alpha$  signaling is vitally important.

HRE-containing genes primarily encode proteins that allow for cell adaptation to low-oxygen environments (Table 1). The net result of increased transcription of target genes is to orchestrate the shift towards anaerobic respiration, to decrease mitochondrial oxygen consumption, and to balance the resulting cellular acidification (34–36). Target genes include enzymes that make up the glycolytic machinery, erythropoietin, which acts

**Table 1. Genes controlled by HIF1 $\alpha$** 

Subtype	Target gene	Function	Reference
Glycolytic machinery	Hexokinase II	Catalyzes the first step in glycolysis	4
	Phosphofruktokinase-1	Rate-limiting enzyme of glycolysis	3, 35
	Glucose-6-phosphate dehydrogenase	Catalyzes first step in the pentose phosphate pathway	91
	Lactate dehydrogenase	Catalyzes lactate production	92
	Pyruvate dehydrogenase kinase	Inhibits PDH-catalyzed production of acetyl-CoA	93
	Glutamate transporter-1	Glucose uptake	94
Inflammatory responses	TLR 2/4	Recognition of PAMPs and DAMPs	95
	IL-1 $\beta$	Proinflammatory cytokine	17
	CXC receptor 4	Chemokine receptor	96
	$\beta$ 2 integrin	Adhesion molecule	97
	ROR $\gamma$ t	Th17 differentiation	22

HRE-containing genes encode for proteins that allow for cell adaptation to low-oxygen environments and inflammatory effector processes.

to increase erythrocyte numbers to promote oxygen transport, and genes involved in angiogenesis such as *VEGF*, which acts to rescue ambient oxygen levels (37, 38). Additionally, there is a whole range of immune-related genes that have HREs in their promoters. In macrophages, pathogen-associated molecular pattern (PAMP) receptors such as certain members of the Toll-like receptor (TLR) family contain an HRE site that results in increased expression in response to HIF1 $\alpha$  stabilization (39). The gene encoding IL-1 $\beta$ , a crucially important cytokine in inflammatory responses, also contains an HRE site in its promoter (17). In Th17 cells, expression of the transcription factor RAR-related receptor  $\gamma$  thymus isoform (ROR $\gamma$ t) is induced by hypoxia. ROR $\gamma$ t promotes proinflammatory Th17 cell differentiation; therefore, HIF1 $\alpha$  can alter the differentiation outcome and thus effector functions of Th17 cells (18, 22). Table 1 lists metabolic and immune-related genes regulated by HIF1 $\alpha$ .

The importance of HIF1 $\alpha$  in the induction of immune-related genes is highlighted by the observation that the metabolic requirements of a resting immune cell and an active immune cell are completely different (40, 41). Therefore, the activating stimuli must also initiate metabolic regulatory events to allow for changing energy needs alongside classic inflammatory responses. Different activators result in different metabolic profiles and HIF1 $\alpha$  provides a switch through which metabolic processes can be reprogrammed. For example, the signals that promote HIF1 $\alpha$  expression in myeloid cells combating pathogens drive a metabolic shift towards decreased oxygen consumption and increased ATP production (42). Many features of HIF-induced metabolic alteration seen in cancer cells have now been observed in active immune cells (42–44). Similarly to cancer cells, this utilization of a Warburg-like metabolism is linked to increased glucose consumption and glycolytic flux. This phenomenon has now been observed in monocytes, macrophages, DCs, and T cells, all cell types with high energy requirements (45).

### HIF1 $\alpha$ in macrophages and DCs

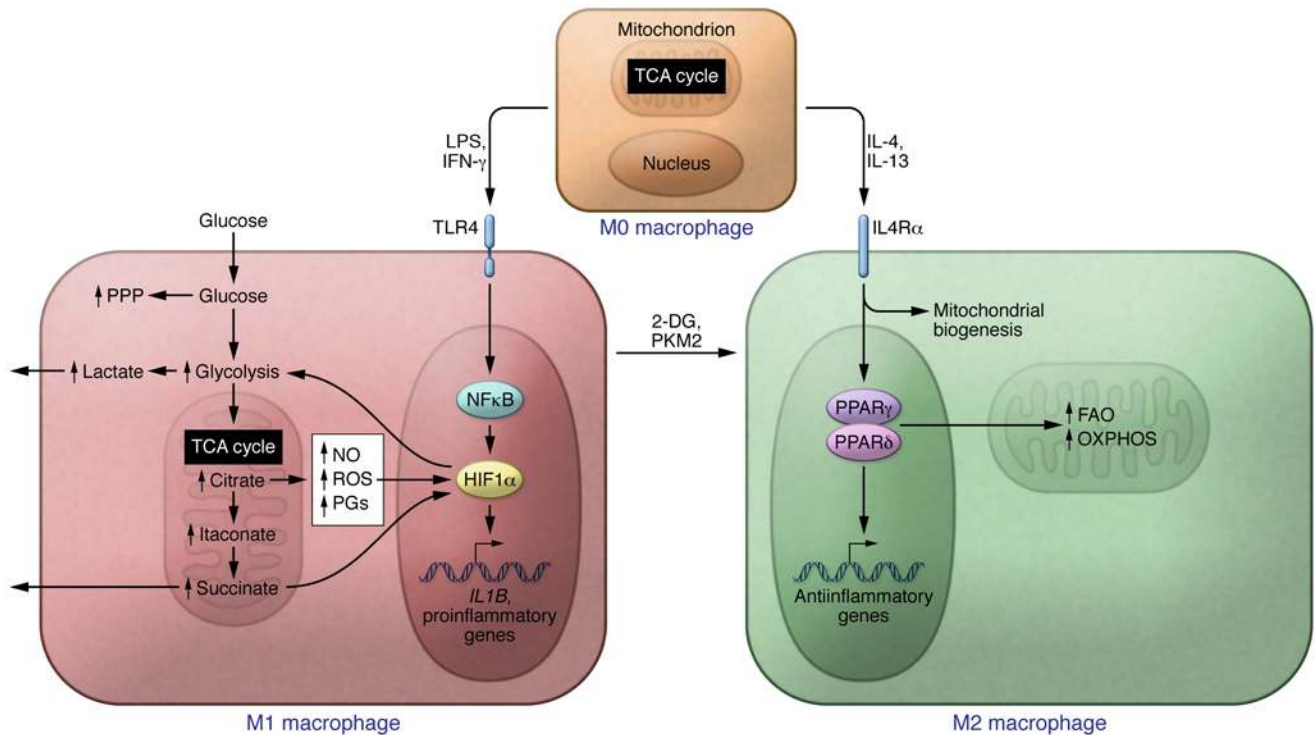
Metabolic reprogramming is crucial to both macrophage and DC activation. DC activation by LPS under normoxia promotes production of HIF1 $\alpha$  mRNA and protein to greater levels than the lev-

els induced by hypoxia alone (20). Inhibition of glycolysis using the glucose analogue 2-deoxyglucose (2-DG) blocks DC maturation in response to LPS, as measured by the reduced expression of the costimulatory molecules CD80 and CD86. HIF1 $\alpha$  is implicated in this process, as the transcription of classic hypoxic response genes such as GLUT1 is increased and HIF1 $\alpha$  deficiency similarly reduces expression of costimulatory molecules (20).

Macrophage activation following LPS stimulation is also dependent on this metabolic shift towards glycolysis. Inhibition of glycolysis with 2-DG blunts the inflammatory response in LPS-treated macrophages. Inhibitors of oxidative phosphorylation have no effect following LPS treatment, indicating that this process is already downregulated by LPS (46). IL-1 $\beta$  production in response to LPS treatment has been shown to be inhibited by 2-DG, and HIF1 $\alpha$  has been implicated in this process (17, 47).

Stimuli such as LPS cause macrophage polarization towards a classically activated phenotype (termed M1), which exhibits inflammatory activities, or an alternatively activated phenotype (termed M2), which exhibits antiinflammatory activities (48). These polarization events are accompanied by HIF-dependent metabolic changes that provide macrophages with the functional ability to respond adequately to their respective stimuli (Figure 1). LPS in combination with IFN- $\gamma$  induces metabolic reprogramming in M1 macrophages via HIF1 $\alpha$ . Metabolism in M1 macrophages is characterized by increased glycolysis and pentose phosphate pathway (PPP) activity. LPS boosts HIF1 $\alpha$  mRNA transcription in phagocytes via induction of NF- $\kappa$ B activity, further increasing signaling through the HIF pathway (49–51). M2 macrophages display a more oxidative metabolic profile compared with M1 macrophages, using oxidative phosphorylation and exhibiting high levels of fatty acid oxidation (FAO) (52, 53). While recent studies have questioned whether FAO has a correlative role or causal role in macrophage polarization (54), it is clear that FAO is essential to energetically support effector functions over a prolonged period of time, as required by M2 macrophages in the defense against parasites and in the resolution phase of infection and injury (55, 56).

Interestingly, HIF1 $\alpha$  and its closely related isoform HIF2 $\alpha$  may exert opposing effects in macrophage polarization. While



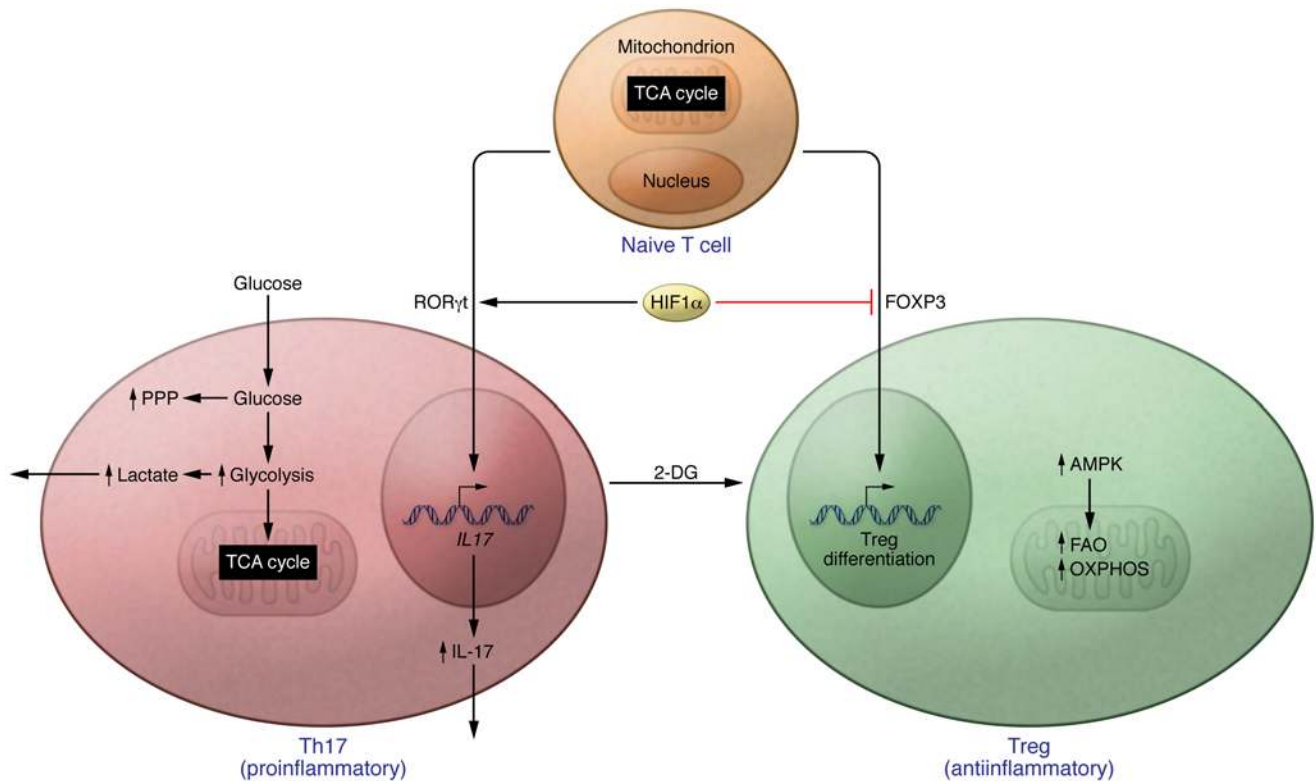
**Figure 1. Metabolic reprogramming in macrophage polarization.** LPS and IFN- $\gamma$  induce M1 macrophages. Metabolism in M1 macrophages is characterized by increased glycolysis and PPP activity, and a broken TCA cycle that leads to metabolite accumulation. M2 macrophages display a more oxidative metabolic profile, with a high reliance on the TCA cycle, utilizing OXPHOS and exhibiting high levels of FAO. Inhibition of glycolysis by 2-DG leads to an oxidative M2 phenotype (48).

depletion of both HIF1 $\alpha$  and HIF2 $\alpha$  is broadly antiinflammatory, HIF2 $\alpha$  does not appear to have a role in the regulation of NO production or the expression of costimulatory molecules. HIF2 $\alpha$  is suggested to be critical for the production of proinflammatory cytokines under hypoxic conditions (19, 57). It has been shown that HIF1 $\alpha$  and HIF2 $\alpha$  are differentially expressed in M1 and M2 macrophages, with HIF1 $\alpha$  induced in M1 macrophages, and HIF2 $\alpha$  increased in M2 macrophages (58). However, in models of sterile inflammation during muscle regeneration, HIF1 $\alpha$  and HIF2 $\alpha$  have been identified as redundant, as macrophage polarization and effector functions were largely unaltered in mice depleted of myeloid HIFs (59).

The importance of HIF1 $\alpha$  in acute innate cell function was dramatically demonstrated by murine HIF1 $\alpha$  deletion studies. Conditional deletion of HIF1 $\alpha$  in the myeloid lineage is not embryonically lethal in a murine model, nor do the mice exhibit any deficiencies in monocyte or neutrophil development and differentiation. However, these mice exhibit impaired inflammatory responses, specifically a defect in cell metabolism that renders the myeloid cells unable to adequately upregulate glycolytic metabolism upon inflammatory stimulation. In the absence of HIF1 $\alpha$ , macrophages exhibit decreased glycolytic rates and energy generation, as well as reduced motility and migration (19). HIF1 $\alpha$  deletion in macrophages causes defects in phagocytic uptake and killing in various bacterial infection models (19, 60). Importantly, mice with myeloid cell-specific deletion of HIF1 $\alpha$  are resistant to LPS-induced lethality, confirming the importance of HIF1 $\alpha$  in a classic *in vivo* model of innate immune activation (61).

An interesting consequence of the recognition of metabolic involvement in innate immune responses has been the identification of certain individual metabolites as signals in inflammation. The nonhypoxic stabilization of HIF1 $\alpha$  in LPS-activated macrophages is mediated by metabolic intermediates with hitherto unknown roles in cell signaling. The TCA cycle intermediates succinate and citrate accumulate following LPS treatment in macrophages (17, 44, 62). Succinate accumulation leads to HIF1 $\alpha$  stabilization and increased transcription of target genes such as *Iilb* (17). The mechanism of succinate accumulation may also involve the metabolite itaconic acid, which is synthesized by immune response gene 1 (IRG1) following citrate accumulation. IRG1 is induced by LPS and converts a citrate derivative, *cis*-aconitate, to itaconic acid. Itaconic acid inhibits an essential metabolic pathway, the glyoxylate shunt, which is necessary for the survival of some pathogenic bacteria (63). Itaconate is also a weak competitive inhibitor of SDH and may contribute to the accumulation of succinate in LPS-treated macrophages (64).

Citrate accumulation is a key event during the metabolic reprogramming process in M1 macrophages, and this reprogramming ultimately impacts HIF1 $\alpha$  stability. Citrate accumulation leads to the production of three important proinflammatory mediators: NO, ROS, and prostaglandins (PGs) (65). Citrate also generates NADPH via pyruvate and malic enzyme. NADPH is required for expression of inducible NOS (iNOS), thereby playing a role in NO production. NO is an important inflammatory mediator that nitrosylates and inhibits components of the electron



**Figure 2. HIF1 $\alpha$  and the Th17/Treg axis.** HIF1 $\alpha$  plays a role in T cell differentiation. HIF1 $\alpha$  directly activates ROR $\gamma$ t transcription and is also involved in the regulation of IL-17 production through association with ROR $\gamma$ t and recruitment of p300 at the *IL17A* promoter. HIF1 $\alpha$  also impedes Treg differentiation by directly binding FOXP3, promoting its proteasomal degradation. Inhibition of glycolysis by 2-DG leads to an oxidative Treg phenotype (22, 72).

transport chain required for OXPHOS. ROS act to stabilize HIF1 $\alpha$  and are produced in a similar fashion by NADPH oxidase (66).

Pyruvate kinase M2 (PKM2) has been shown to be a critical determinant of metabolic reprogramming in macrophages via HIF in response to LPS stimulation. PKM2 is the rate-limiting enzyme of glycolysis that converts phosphoenolpyruvic acid (PEP) to pyruvate. Enzymatically inactive PKM2 monomers or dimers exist in equilibrium with enzymatically active PKM2 tetramers. PKM2 dimers can translocate to the nucleus, where they directly interact with HIF1 $\alpha$  to regulate expression of proglycolytic enzymes (67). The highly active PKM2 tetramers are retained in the cytosol, supporting the final step of glycolysis (68). PKM2 is upregulated in tumors and LPS-activated macrophages (69, 70). Following LPS activation of macrophages, PKM2 dimers stabilize HIF1 $\alpha$ , thereby regulating HIF1 $\alpha$  target genes such as *Il1b* and genes encoding the glycolytic machinery. Forcing PKM2 into its tetrameric form using the small-molecule activator DASA-58 or TEPP-46 prevents nuclear translocation. This impairs interaction with HIF1 $\alpha$  and reverses the LPS-induced, HIF1 $\alpha$ -mediated shift towards glycolysis and IL-1 $\beta$  production, reprogramming macrophages to an M2-like phenotype (69). These findings support the importance of the PKM2/HIF1 $\alpha$  axis for M1 macrophage differentiation and function, with PKM2/HIF1 $\alpha$  acting as a pivot in the process of M1/M2 differentiation.

It is important to note that the role of HIF in macrophages and other immune cells is neither definitive nor without contro-

verses. The HIF signaling pathway modulates a significant number of crucial cellular processes and must be carefully controlled; thus, it follows that there exist multiple levels of regulation, including positive and negative feedback loops as well as extensive crosstalk with other signaling pathways. Responses may be dependent on time and context. For example, there are important differences in the regulation of HIF by hypoxia versus regulation by inflammatory stimuli such as LPS. HIF is required for the later shift to glycolysis that occurs in DCs, but the immediate metabolic switch is HIF independent and is mediated by the PI3K/Akt pathway (20). Many aspects have yet to be elucidated, but the primary model of HIF-mediated regulation is a multilevel regulatory network of great complexity that modulates responses such as proliferation, apoptosis, and differentiation (71).

### HIF1 $\alpha$ and the Th17/Treg balance

Th17 cells are a potent, proinflammatory subset of the T helper cell family and are defined by their characteristic production of the proinflammatory cytokine IL-17. Th17 cells are required during the response to some pathological stresses, but exhibit immunopathological functions in experimental and naturally occurring autoimmune settings. Upon activation, naive T cells undergo exponential clonal expansion and experience a dramatic surge in bioenergetic demands. Similarly to M1 macrophages, increased energy requirements following Th17 differentiation are compensated for by a switch to a glycolytic phenotype in

order to sustain ATP levels and support the biosynthetic needs of the rapidly dividing cells. This metabolic reprogramming occurs in a Warburg-like fashion, independently of oxygen levels (22, 72). The shift is primarily orchestrated by an mTOR-dependent nutrient-sensing pathway (73).

The inhibition of glycolysis by 2-DG abrogates Th17 development (72). Inhibition or knockdown of pyruvate dehydrogenase kinase (PDHK) indirectly fosters glycolysis by blocking pyruvate dehydrogenase (PDH) to suppress pyruvate oxidation and promote its conversion to lactate, which also inhibits Th17 differentiation (18). The blockade of acetyl-CoA carboxylase 1 (ACC1), a crucial enzyme in fatty acid synthesis, also limits Th17 proliferation and attenuates Th17-mediated pathologies (74). Pharmacological inhibition of glycolysis and glutaminolysis and promotion of FAO reduced the proliferation of lymphocytes, particularly effector T cells, in a transplantation model (75).

HIF1 $\alpha$  is implicated in the Th17 cell differentiation process through its direct activation of ROR $\gamma$ t and regulation of IL-17 production through association with ROR $\gamma$ t at the *Il17a* promoter to recruit the transcriptional coactivator p300 (22). Under conditions favoring Th17 differentiation, HIF1 $\alpha$  is upregulated in a STAT3-dependent manner. Murine studies have shown that HIF1 $\alpha$  deficiency specifically impedes Th17 development and protects mice from experimental autoimmune encephalitis, further implicating HIF1 $\alpha$  in Th17 differentiation and effector functions in a classic model of Th17-dependent pathology (22).

Tregs are classified as professional suppressors of varied immune responses and inflammatory processes, and central keepers of self tolerance (76). Tregs functionally oppose Th17 cells by producing the antiinflammatory cytokine IL-10. Deficiency in Tregs leads to systemic autoimmunity in mouse models and in patients (termed immunodysregulation polyendocrinopathy enteropathy X-linked) (77). Treg function is intricately linked to their metabolic profile, where FAO predominates (40).

HIF1 $\alpha$  impedes the development of Tregs by directly binding FOXP3, a crucial transcription factor in Treg development, and targets it for ubiquitination and subsequent proteasomal degradation (Figure 2) (22). Loss of VHL results in excessive production of IFN- $\gamma$  by Tregs, leading to induction of a Th1-like phenotype and an inability to prevent the induction of colitis. Silencing of HIF1 $\alpha$  in Tregs reverses these proinflammatory effects and restores their regulatory function (78). This direct modulation of the TH17/Treg balance marks HIF1 $\alpha$  as an important director of T cell fate and immune effector functions (22, 79).

### HIF1 $\alpha$ in neutrophils

Neutrophils are short-lived granulocytes that are crucial for the defense against microbes. They induce pathogen killing via ROS, NO, and phagocytosis (80), and on occasion the generation of extracellular traps (81).

Metabolic reprogramming in response to immune stimuli has long been described in neutrophils. The Warburg-like shift toward a more glycolytic phenotype was first observed in neutrophils in 1959 (43). Stimulation with LPS induces an increase in glucose uptake and oxygen consumption by neutrophils. Increased oxygen consumption is required for the production of H<sub>2</sub>O<sub>2</sub> for pathogen clearance (82). HIF signaling and expression

of the PHD enzymes, specifically PHD3, have been implicated in the prolonged survival of neutrophils in hypoxic conditions (83).

Interestingly, neutrophil-driven hypoxia in the microenvironment has been shown to be antiinflammatory in a model of severe colitis. Neutrophil activation and migration rapidly depletes local oxygen and leads to localized induction of HIF signaling in epithelial cells, increasing barrier function and preventing further infiltration of proinflammatory immune cells. Further, mice lacking a neutrophil respiratory burst developed severe colitis, which was attenuated by stabilizing HIF in the mucosa (84). The intestinal epithelium is characterized by a steep gradient in cellular oxygen tension, in which the tips of the villi are hypoxic and oxygenation increases with distance from the intestinal lumen. Dysregulated oxygen tension is a feature of inflammatory bowel disease (IBD) (85, 86). Expression of HIF1 $\alpha$  and HIF2 $\alpha$  is augmented in epithelial cells in ulcerative colitis and Crohn's disease patients (25). Increased HIF1 $\alpha$  expression enhances epithelial barrier function and antimicrobial defense, whereas increased HIF2 $\alpha$  expression activates inflammatory cytokines to stimulate epithelial proliferation for inflammatory resolution and tissue regeneration (25, 87).

### Hypoxic signaling and additional metabolic pathways

Recent work in the field has illustrated an additional role for hypoxic signaling in regulation of additional metabolic pathways that may be important in immune regulation. Amino acid metabolism such as the catabolism of tryptophan has been shown to be affected by the inhibition of PHD enzymes. Inhibition of PHD2 results in the accumulation of  $\alpha$ -ketoglutarate which in turn boosts hepatic kynurenic acid production (88). Kynurenic acid has been shown to be protective in certain models of tissue injury such as cardiac ischemic protection (88-90).

### Conclusions and future perspectives

The observation that succinate can modulate HIF1 $\alpha$  activation in macrophages and that HIF1 $\alpha$  is critical for macrophage function has resulted in the recognition of the roles played by metabolites outside their classical function as metabolic pathway intermediates. This presents an exciting frontier in immunology, as the roles of metabolites expand to include mediation of immune responses. The metabolic changes that underlie innate immune cell function and, more importantly, the pathways that induce metabolic reprogramming leading to altered immune cell phenotypes represent tangible new therapeutic targets for immune modulation.

The delineation of cellular subsets by their primary metabolic pathways will be critical in therapeutic targeting for the treatment of certain pathologies. For example, Th17 cells play a pathogenic role in many autoimmune pathologies and represent an attractive target for therapy. Distinct, targetable metabolic differences are exhibited by proinflammatory Th17 cells and Tregs. Pharmacological promotion of Treg differentiation in certain Th17-driven models such as experimental autoimmune encephalitis, for example via impairment of glycolysis by administration of 2-DG, has proven promising (72). Skin and heart allograft rejection can be prevented by inhibiting glycolysis with

2-DG, glutamine metabolism with the glutamine analog DON, and augmenting FAO with metformin (75). Thus, there are multiple pharmacological modalities that could potentially be used to alter immune metabolic programs.

The HIF pathway is critically involved in both the adaption to environmental changes such as alterations in oxygen level and in governing immune responses. The pathogenesis of several conditions, including RA and IBD, result from the simultaneous deregulation of both inflammatory and hypoxia response pathways. A further consideration is that hypoxic signaling has pleiotropic effects on additional metabolic pathways that may be important in immune regulation. Dissection of the meta-

bolic regulation of and crosstalk between these pathways is an important research goal that may lead to the development of novel therapeutic strategies.

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