

# Hypoxia and the regulation of myeloid cell metabolic imprinting: consequences for the inflammatory response

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## Abstract

Inflamed and infected tissue sites are characterised by oxygen and nutrient deprivation. The cellular adaptations to insufficient oxygenation, hypoxia, are mainly regulated by a family of transcription factors known as hypoxia-inducible factors (HIFs). The protein members of the HIF signalling pathway are critical regulators of both the innate and adaptive immune responses, and there is an increasing body of evidence to suggest that the elicited changes occur through cellular metabolic reprogramming. Here, we review the literature on innate immunometabolism to date and discuss the role of hypoxia in innate cell metabolic reprogramming, and how this determines immune responses.

**Keywords** hypoxia-inducible factors; hypoxia; immunometabolism; macrophages; neutrophils

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See the Glossary for abbreviations used in this article.

## Introduction

A key feature of immune cells is their ability to infiltrate and function in hypoxic tissues, enabling appropriate responses to damage and infection. Hypoxia primes innate cells for the inflammatory response and prolongs survival in numerous types of innate immune cells including neutrophils, monocytes and eosinophils [1–4]. HIF-mediated innate immune responses are discussed in detail in a review by Harris *et al* [5]. Both hypoxia and the activation of innate cells by inflammatory stimuli result in the induction of the master regulator of oxygen homeostasis, hypoxia-inducible factor-1 (HIF-1) [6]. HIF-1 induction mediates a response through the regulation of expression of hundreds of downstream genes involved in diverse biological pathways, ranging from erythropoiesis to metabolism [7–9]. In mammals, HIF-1 exists as a heterodimeric complex composed of two subunits,

HIF-1 $\alpha$  (oxygen-sensitive) and HIF-1 $\beta$  (constitutive) [10,11]. Hypoxia-inducible factor activity is regulated by a family of oxygen sensing proteins known as prolyl hydroxylase domain enzymes (PHD1-3) and asparaginyl hydroxylase enzyme factor inhibiting HIF (FIH) [11–14]. Prolyl hydroxylase domain enzymes post-translationally modify the HIF-1 $\alpha$  subunit through hydroxylation of two conserved proline residues, in an oxygen-dependent manner. Hydroxylation destabilises HIF-1 $\alpha$  by mediating binding to von Hippel Lindau (VHL) protein E3 ligase complex followed by ubiquitination and proteasomal degradation [15,16]. In order to function, in addition to oxygen, PHD and FIH proteins also require Fe<sup>2+</sup>, ascorbic acid and the tricarboxylic acid (TCA) cycle intermediary  $\alpha$ -ketoglutarate [17]. Furthermore, these proteins have been shown to be inhibited by the accumulation of downstream TCA intermediaries succinate and fumarate in studies using cancer cells and *in vitro* studies where cells were exposed to high concentrations of TCA cycle intermediates [18,19]. These findings highlighted a direct link between metabolite availability and the regulation of HIF-1 activity. To date, there are a limited number of studies investigating metabolic regulation via TCA cycle metabolites under physiological oxygen conditions in myeloid cells *in vivo*. In hematopoietic stem cells, fumarate breakdown has been identified as a critical regulatory mechanism for stem cell maintenance and hematopoietic differentiation in normal hematopoiesis and leukaemia propagation [20]. A second study by Tyrakis *et al* [21] demonstrated the accumulation of the metabolite 2-hydroxyglutarate in mouse CD8<sup>+</sup> T cells in response to T-cell receptor triggering and showed that it occurs through a HIF-1 $\alpha$ -dependent mechanism with subsequent epigenetic changes. In *in vitro* assays utilising activated macrophages, succinate accumulation has been shown to be important in the regulation of downstream cellular responses [22]. It remains to be seen whether other TCA cycle metabolites act as immunoregulatory molecules in neutrophils and macrophages in normal physiology and during inflammatory hypoxia in the tissues.

In this review, we focus on the effects of hypoxia on neutrophil and macrophage metabolic regulation. For a more general overview of the HIF pathway relevance for metabolism in all immune cells, see the review by Krzywinska and Stockmann [23].

**Glossary**

<b>2-DG</b>	2-deoxyglucose
<b>Ap3A</b>	diadenosine triphosphate
<b>ATP</b>	adenosine triphosphate
<b>CARKL</b>	carbohydrate kinase-like protein
<b>Cx43</b>	connexin 43
<b>DCs</b>	dendritic cells
<b>FADH<sub>2</sub></b>	flavin adenine dinucleotide
<b>FIH</b>	factor Inhibiting HIF
<b>G6P</b>	glucose-6-phosphate
<b>G6PT</b>	glucose-6-phosphate (G6P) transporter
<b>GAPDH</b>	glyceraldehyde 3-phosphate dehydrogenase
<b>GSD-1b</b>	glycogen storage disease type 1b
<b>HID</b>	hyper-IgD syndrome
<b>HIF</b>	hypoxia-inducible factor
<b>LPS</b>	lipopolysaccharide
<b>NADH</b>	nicotinamide adenine dinucleotide
<b>NADPH</b>	nicotinamide adenine dinucleotide phosphate
<b>NETs</b>	neutrophil extracellular traps
<b>NO</b>	nitric oxide
<b>NOX2</b>	NADPH oxidase 2
<b>OPA1</b>	optic atrophy 1
<b>PDH</b>	pyruvate dehydrogenase
<b>PDK1</b>	pyruvate dehydrogenase kinase 1
<b>PHDs</b>	prolyl hydroxylase domain enzymes
<b>PKM2</b>	pyruvate kinase M2
<b>PNS</b>	peripheral nervous system
<b>PPP</b>	pentose phosphate pathway
<b>ROS</b>	reactive oxygen species
<b>TAMs</b>	tumour-associated macrophages
<b>TCA</b>	tricarboxylic acid cycle
<b>VHL</b>	von Hippel Lindau

**Regulation of metabolism by hypoxia**

For adequate function, innate immune cells require readily available ATP, redox buffering capacity and biosynthetic precursors. Under normoxic oxygen levels, most cells metabolise glucose into pyruvate via glycolysis. Pyruvate then enters the mitochondria and can be converted into acetyl-coenzyme A, which is further oxidised in the TCA cycle. This process, known as aerobic respiration, generates the reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH<sub>2</sub>), which donate electrons to the electron transport chain and fuel oxidative phosphorylation. Aerobic respiration generates high levels of energy stores in the form of adenosine triphosphate (ATP) molecules. It was initially thought that hypoxic cells relied solely on anaerobic glycolysis, a phenomenon termed the “Pasteur effect” [24]. This phenomenon was used to describe the elevated conversion of glucose to lactate as a result of HIF-activated glycolysis and was thought to be a result of a decline in aerobic respiration, a passive process resulting from oxygen deprivation. However, mounting evidence has revealed that hypoxia actively regulates metabolic pathways, and in addition to upregulating glycolytic flux, it also suppresses the TCA cycle and the mitochondrial respiratory chain [25]. A study by Kim *et al* [26] has demonstrated that mitochondrial respiration in hypoxic cells causes leakage of electrons in the transport chain leading to increased reactive oxygen species (ROS) levels and cell damage. Pyruvate dehydrogenase (PDH) inhibition through HIF-1-mediated pyruvate

dehydrogenase kinase 1 (PDK1) induction was shown to prevent cell damage by attenuating mitochondrial respiration and shunting metabolism to glycolysis. This subsequently results in the maintenance of ATP levels and restored homeostasis.

**The impact of hypoxia on immunometabolism**

Innate immune cells eradicate pathogens and convey signals to the adaptive immune system in turn regulating its response. Innate cells may be resident cells or cells that migrate through a range of oxygen tensions before reaching very hypoxic environments such as inflamed or infected tissues. These cells therefore require oxygen-independent mechanisms of energy generation. Hypoxia results in the activation of numerous innate immune cells, and the HIF pathway has been shown to play key roles in both effective and pathological immunity [27]. Hypoxia-inducible factor activation can occur under normoxia, and this allows the initiation of an inflammatory response before the tissue becomes hypoxic. Gram-negative bacterial product lipopolysaccharide (LPS), ROS and reduced cellular iron have all been shown to regulate HIF-1 expression under normoxic conditions through upregulation of *HIF-1 $\alpha$*  transcript expression, HIF-1 $\alpha$  stabilisation or inhibition of PHDs, respectively [28–30].

**Neutrophils**

Neutrophils constitute around 60% of the circulating leucocytes. They are short-lived polymorphonuclear cells and the first to migrate to injured or infected tissue sites where oxygen availability is limited. Their role is to combat bacterial infections through phagocytosis, respiratory burst activity, the release of granule contents and extracellular traps. Hypoxia prolongs neutrophil survival by inhibiting programmed cell death and is accompanied by a time-dependent induction of key glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and triosephosphate isomerase-1 [1]. Murine studies on *HIF-1 $\alpha$*  knockouts have demonstrated that the HIF-1 $\alpha$  protein is essential for myeloid cell infiltration and activation. HIF-1 $\alpha$  inactivation results in a reduced cellular ATP pool, impairment of myeloid cell aggregation, motility, invasiveness and bacterial killing [31,32]. Although HIF-2 $\alpha$  is also upregulated in neutrophils in response to hypoxia, it is thought to play a key role in the resolution phase of inflammation and its effects on metabolism are not understood [33].

Neutrophils contain very few mitochondria and as such are thought to lack the capacity for mitochondrial respiration [34]. In keeping with this, it has been shown that the inhibition of oxidative phosphorylation has little effect on the oxygen consumption rate of neutrophils [35]. Neutrophils rely heavily on glycolysis even in the presence of oxygen [36,37]. In keeping with this, neutrophil activation results in increased glucose transport and intrinsic activation of glucose transporter molecules [38].

Glycolysis provides a very rapid supply of energy and is well suited to the function of recruited innate immune cells. As early as in the 1950s, Borregaard and Herlin demonstrated that glycolysis is used to fuel neutrophil phagocytosis in both the presence and absence of glucose [39]. The ATP required for the phagocytosis of zymosan particles was almost exclusively generated through either glucose uptake or glycogenolysis in a glucose-deplete setting. This

finding was further supported by a study investigating the inhibition of glycolysis by 2-deoxyglucose (2-DG) on guinea pig neutrophils, where treatment of cells with the inhibitor led to an impairment of the phagocytosis of C3- and IgG-bound particles [40]. The effects of the metabolic substrates fatty acids and glutamine have been studied to a lesser extent, but both have also been shown to play roles in neutrophil development and homeostasis. While autophagy-derived free fatty acids and their oxidation are required for appropriate neutrophil differentiation, the role of glutamine is critical in regulating inflammatory processes through delaying spontaneous apoptosis [41,42].

A shunt into the pentose phosphate pathway (PPP), a side pathway of glycolysis, ensures that the neutrophil has a supply of nicotinamide adenine dinucleotide phosphate (NADPH) cofactor for respiratory burst via NADPH oxidase 2 (NOX2) activity [43]. The importance of the pentose phosphate pathway in neutrophil reactive oxygen species production has been established in studies on patients with glucose-6-phosphate dehydrogenase deficiency, who have abnormal neutrophil function and increased susceptibility to infections [44]. These patients have neutrophils with very low levels of NADPH, unable to produce normal quantities of superoxide species. *In vitro*, inhibition of the pentose phosphate pathway using the 6-phosphogluconate-dehydrogenase inhibitor 6-aminonicotinamide abrogated bacterial peptide fMLF and phorbol myristate acetate induced NADPH production and superoxide production [45].

Neutrophil activation results in the production of extracellular traps or fibres consisting of granule proteins and chromatin [46]. Neutrophil extracellular traps (NETs) degrade virulence factors and kill bacteria. It has been reported that NETosis, the form of cell death characterised by the release of NETs, is dependent on NADPH oxidase, as patients with chronic granulomatous disease who carry mutations in NADPH oxidase are unable to produce NETs [47]. Conversely, NETs have also been implicated in the pathogenesis of autoimmune and inflammatory disorders [48]. The formation of neutrophil extracellular traps is heavily dependent on glucose substrate and to a lesser extent on glutamine [49]. Moreover, a study by Azevedo *et al* [50] observed that a shift to the pentose phosphate pathway and not mitochondrial ROS is necessary for the generation of NETs. Although mitochondrial ROS are not effective in producing NETs, a very recent study claims that the mitochondrial inner membrane protein optic atrophy 1 (OPA1) is important for the anti-microbial defence of neutrophils in an *in vivo* model of *P. aeruginosa* lung infection [51]. OPA1 was shown to function through the control of the mitochondrial electron transport complex I and in turn regulate NAD<sup>+</sup> availability. NAD<sup>+</sup> is required for the enzymatic activity of the glycolytic protein glyceraldehyde-3-phosphate dehydrogenase. Opa1-deficient neutrophils had lower NAD<sup>+</sup> levels resulting in a reduced glycolytic rate and a decline in ATP production.

In addition to increased uptake and affinity for glucose during neutrophil activation, neutrophils also contain and upregulate glucose stores in the form of the macromolecule glycogen [38,52–54]. Hypoxia is known to promote glycogen accumulation through HIF-1-mediated induction of glycogen synthase 1 [55]. We have recently demonstrated the consequences of HIF-1 $\alpha$  stabilisation for neutrophilic inflammation. Our work has shown that PHD2-deficient neutrophils have enhanced survival, chemotaxis and functional

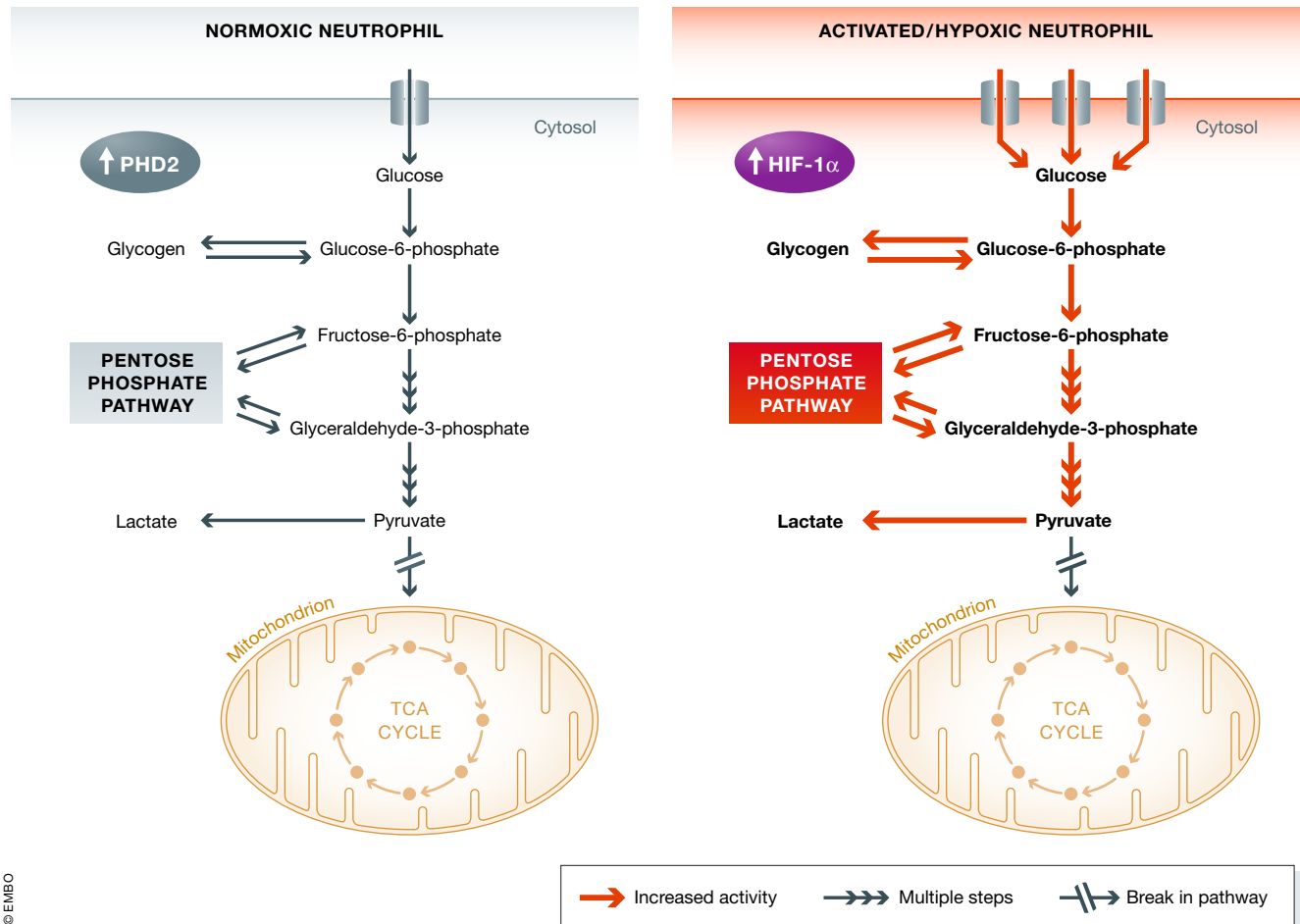
reserve capacity resulting in an exaggerated inflammatory response that is detrimental to the host [56]. This disordered neutrophilic response is driven by increases in both glycolytic flux and glycogen storage. Importantly, non-PHD-isoform-selective inhibition using Molidustat also replicated the Phd-2-deficient phenotype augmenting neutrophilic inflammation via increased extracellular acidification rate (an indirect measure of glycolysis) and ATP levels [56]. With numerous PHD protein inhibitors being used for the pharmacological treatment of anaemia associated with chronic disease and in late phase clinical trials [57], understanding the consequences of pan hydroxylase inhibition for immunometabolism and therefore innate cellular responses will be crucial for determining when and how these inhibitors are administered to limit adverse effects.

More broadly, the importance of glucose homeostasis for neutrophil function is supported by study of patients with glycogen storage disease type Ib (GSD-Ib). In this setting, defective glucose-6-phosphate (G6P) transporter (G6PT) activity is associated with impaired neutrophil respiratory burst, chemotaxis and calcium mobilisation, resulting in an increased susceptibility to infection [58–60]. Furthermore, mice lacking glucose-6-phosphatase-b (G6Pase-b) also demonstrate an increased susceptibility to bacterial infection with elevated neutrophil apoptosis responses as a consequence of dysregulated activation of the HIF-1 $\alpha$ /PPAR- $\gamma$  pathway [59,60]. Together therefore, these data highlight the importance of the tight regulation of intracellular glucose shunting for effective neutrophil responses.

Besides the intracellular metabolic regulation, neutrophils have also been reported to release metabolites to the external environment. It has long been known that neutrophils release both AMP and ATP at inflammatory sites [61,62]. Adenosine triphosphate is released both through connexin 43 (Cx43) hemichannels and through vesicular nucleotide transporters [62,63]. ATP secretion results in autocrine stimulation of these cells through purinergic receptors. Additionally, it directs the cell orientation and is rapidly hydrolysed [64]. There is some evidence from *in vitro* models of endothelial barrier function and neutrophil-endothelial adhesion to indicate that neutrophils secrete ATP in response to hypoxia and inflammation [65]. In this setting, it was demonstrated that neutrophil-derived ATP signals through endothelial adenosine receptors to promote endothelial barrier function and attenuates PMN-endothelial adhesion [65]. In a second study by Eltzschig *et al* [66], endogenous neutrophil adenosine produced during hypoxia was shown to reduce neutrophil accumulation implicating this pathway as a mechanism for attenuating excessive neutrophil inflammation. More recently, there is novel evidence for activated neutrophils acting as a source of diadenosine triphosphate (Ap3A) and ensuring an additional supply of adenosine during mucosal inflammation resolution [67]. These studies highlight the metabolic crosstalk between neutrophils and other tissues and provide support for the role of neutrophils in regulating barrier formation and wound healing responses. A summary diagram of the metabolic changes occurring during exposure to hypoxia and activation of neutrophils is shown in Fig 1.

### Macrophages

Monocytes are short-lived mononuclear phagocytes circulating in the bloodstream. They contribute to and replenish tissue-resident phagocytes by giving rise to monocyte-derived macrophages and



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**Figure 1. Neutrophil metabolic changes during exposure to hypoxia and activation.**

Under normoxic conditions (left), neutrophils express high levels of PHD2 which result in the targeting of HIF-1 $\alpha$  for proteasomal degradation. In this setting, neutrophil homeostasis relies on the glycolytic pathway. Neutrophils further increase both their glycolytic and pentose phosphate pathway flux in response to hypoxic culture and stimulation (right). In addition, they also upregulate the influx of extracellular glucose and glycogen storage. The neutrophil TCA cycle is a dysfunctional cycle with only partial activity.

dendritic cells (DCs). Macrophages play a central role in the innate immune response and are characterised by considerable functional diversity and plasticity [68]. These cells are recruited to sites of injury or infection to either promote or reduce inflammation through the phagocytosis of bacteria and efferocytosis [69]. In addition, they help to repair the tissue damage caused by the innate immune response. Macrophage accumulation occurs in numerous hypoxic tissues such as arthritic joints, atherosclerotic plaques and malignant tissues, implicating these cells in these diseases. Both human monocytes and macrophages markedly upregulate HIF-1 $\alpha$  levels during *in vitro* exposure to hypoxic environments as well as to LPS exposure [70,71]. Furthermore, human macrophages have also been shown to upregulate their HIF-2 $\alpha$  levels under hypoxia [71]. HIF-1 $\alpha$  deficiency in macrophages displays defects in key cellular functions including motility, aggregation and invasion [31]. By contrast, HIF-2 $\alpha$ -deficient macrophages lose 50% of their migratory and invasive capacity [72].

Over 40 years ago, Hard made the observation that activated macrophages were more glycolytic and had a decreased oxygen

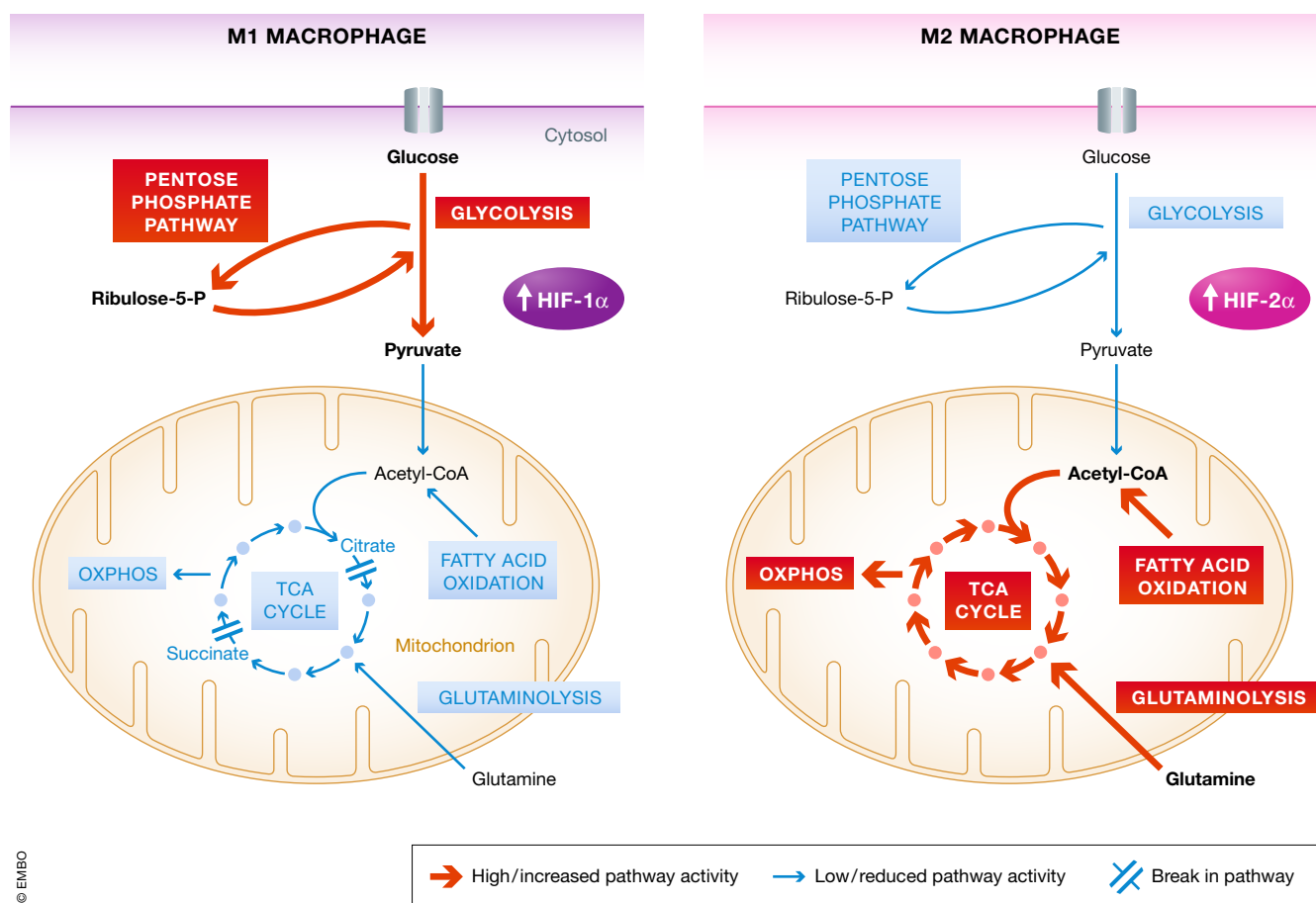
consumption rate [73]. Twenty years later, in a study by Newsholme *et al* [74] it was shown that most of the glucose in activated macrophages is utilised for conversion into lactic acid and a very low level was being used for oxidative phosphorylation. These findings were paralleled by increases in the expression of glycolytic enzyme hexokinase and the PPP enzyme glucose-6-phosphate dehydrogenase [75].

Activated macrophages are commonly divided into two polarised states or phenotypes, the classically activated M1 and the alternatively activated M2 [76,77]. M1-polarised macrophages are reported to have induced HIF-1 $\alpha$  expression, whereas M2 macrophages demonstrate upregulated HIF-2 $\alpha$  levels [78]. In keeping with this finding, myeloid-specific overexpression of HIF-1 $\alpha$  in mice resulted in a hyper-inflammatory state characterised by the upregulation of M1 markers and was associated with increased glycolytic activity [70]. As with neutrophils, the PHD2 isoform is the main regulator of the glycolytic reprogramming of macrophages demonstrated by gene knockout studies in mice [79]. A role for PHDs in macrophage function was demonstrated in a hind-limb ischaemia study, where PHD2

haploinsufficiency in mice was sufficient to skew macrophages to an M2 phenotype without affecting HIF-1 $\alpha$  levels leading to a pro-arteriogenic phenotype in turn preventing hind-limb ischaemia in a murine model [80,81].

Over the last few years, it has become increasingly apparent that the two divergent macrophage phenotypes are associated with differing metabolic states. M1 polarisation results from activation of interferon- $\gamma$  or toll-like receptor activation by LPS. In this setting, both macrophages and dendritic cells increase the expression of the nitric oxide synthase generating large amounts of nitric oxide, which inhibits mitochondrial respiration and is utilised for bacterial killing [81,82]. In contrast, M2 polarisation is a consequence of exposure to IL-4 or IL-13 [68]. The M2 phenotype is characterised by fatty acid oxidation and mitochondrial biogenesis via STAT6 and PGC-1 $\beta$  and reduced nitric oxide levels [83,84]. Inhibition of the mitochondrial respiratory chain in M2 macrophages not only blocks the M2 phenotype but also drives the cells to an M1 phenotype [83]. In addition, M2 macrophages have upregulated levels of arginase 1 driven by HIF-2 $\alpha$  [78,85]. Arginase 1 is an enzyme, which competes with inducible nitric oxide for the common substrate L-arginine.

Macrophage activation via LPS exposure induces dramatic metabolic reprogramming. As mentioned earlier, M1 macrophages undergo a switch from generating ATP by oxidative phosphorylation to glycolysis. The dramatic changes in the metabolic activity of M1 macrophages are also regulated by the attenuation of pyruvate kinase M2 (PKM2) levels. Activated PKM2 can bind to HIF-1 $\alpha$  and prevent it from inducing the IL-1 $\beta$  promoter activity [22]. The metabolic shift in M1 macrophages resembles the Warburg effect first reported in tumour cells and is associated with increased levels of the TCA cycle intermediary succinate due to high glutaminolysis activity [86,87]. The TCA cycle in these cells is broken at two points: after citrate and after succinate [88]. The effects of succinate are exerted in three ways, firstly by inhibiting PHD proteins and thus leading to the stabilisation of HIF-1 $\alpha$  [89]. Secondly, the elevated levels of succinate result in increased protein succinylation states, glycolytic targets of which include glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and malate dehydrogenase [87]. It is not known whether this affects the enzymatic activity of these proteins. Thirdly, the oxidation of succinate in M1 via succinate dehydrogenase macrophages generates mitochondrial ROS and it



**Figure 2. Macrophage metabolic profiles of M1- and M2-polarised cells.**

M1 activated macrophages are reported to have elevated HIF-1 $\alpha$  expression associated with an increase in the utilisation of the glycolytic and pentose phosphate pathway (left). Conversely, these cells demonstrate a reduced activity of mitochondrial respiration with the TCA cycle being broken at two points: after citrate and after succinate. Alternatively, activated M2 macrophages (right) show upregulated HIF-2 $\alpha$  expression and are characterised by increased fatty acid oxidation and mitochondrial respiratory chain activity.

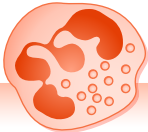
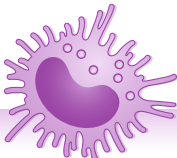
has been reported that this is crucial for determining the inflammatory phenotype [90].

There is evidence to suggest that macrophage polarisation can be influenced by changes in glucose metabolism and PPP activity [91,92]. The carbohydrate kinase-like protein (CARKL) was identified as a non-protein kinase that is rapidly downregulated upon LPS stimulation in mice and humans and directs carbon reshuffling between glycolysis and the PPP. It was shown to play an important role in sensitising macrophages to an M2 polarisation by shifting glucose utilisation from the PPP to glycolysis. In the M1 macrophages on the other hand, elevated PPP activity generates NADPH important both for ROS and for NO synthesis [93]. Another M1-regulated metabolic switch occurs through increased expression of PFKFB3, an isoform of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2), which increases the levels of the metabolite fructose-2,6-bisphosphate [94,95]. This metabolite acts by activating the glycolytic enzyme 6-phosphofructo-1-kinase. In addition, the attenuated levels of fatty acid oxidation and mitochondrial respiratory chain activity in both dendritic cells and M1 macrophages are achieved through the inhibition of AMPK [96,97]. A summary

diagram showing the regulation of different metabolic pathways in M1 and M2 macrophages is shown in Fig 2.

Substrate availability can also regulate macrophage polarisation. Mechanistic target of rapamycin (mTOR) signalling has been linked to macrophage polarisation and is likely to play a role in regulating responses to substrate availability [98]. For example, both mTORC1 and Lamtor1, a lysosomal adaptor protein which forms an amino acid sensing complex with lysosomal vacuolar-type H<sup>+</sup>-ATPase and is the scaffold for amino acid-activated mTORC1, were shown to be required for M2 polarisation [99]. Jha *et al* [88] also demonstrated that glutamine deprivation can negatively affect M2-polarised macrophages but not M1. This was linked to the glutamine deprivation changes exerted on the TCA cycle. Moreover, the UDP-GlcNac biosynthesis pathway was also highlighted as being important for M2 activation and was proposed by the authors to function by facilitating protein folding [88].

Several studies have reported the influence of hypoxia and HIF expression on macrophage responses. For example, hypoxia has been shown to improve the control *Leishmania amazonensis* infection [100]. Both HIF-1 $\alpha$  and HIF-2 $\alpha$  were shown to be upregulated

	NEUTROPHILS 	MACROPHAGES / DCs 	
<b>HIF response</b>	<b>↑ Hif-1<math>\alpha</math></b>	<b>↑ Hif-1<math>\alpha</math></b>	<b>↑ Hif-2<math>\alpha</math></b>
<b>Metabolic phenotype</b>	<b>M1 polarisation/ LPS activated DCs</b> <ul style="list-style-type: none"> <li>↑ Glucose uptake</li> <li>↑ Glycolysis</li> <li>↑ Glycogen stores</li> <li>↓ OxPhos</li> <li>↑ ATP release</li> </ul>	<b>M2 polarisation</b> <ul style="list-style-type: none"> <li>↑ OxPhos</li> <li>↑ Fatty acid oxidation</li> <li>↑ PPP</li> <li>↓ L-arginine</li> <li>↓ NO</li> <li>↑ UDP-GlcNac</li> </ul>	
		<ul style="list-style-type: none"> <li>↑ Glucose uptake</li> <li>↑ Glycolysis</li> <li>↓ PPP</li> <li>↓ OxPhos</li> <li>↑ NO</li> <li>↑ ROS</li> <li>↑ Glutaminolysis</li> <li>↑ Succinate</li> </ul>	
<b>Outcomes</b>	<b>PHD2 inactivation</b> <ul style="list-style-type: none"> <li>→ Excessive inflammation</li> <li>→ Tissue injury</li> </ul> <b>Acute hypoxaemia and infection</b> <ul style="list-style-type: none"> <li>→ Increased morbidity and mortality</li> </ul> <b>Hypoxic preconditioning prior to infection</b> <ul style="list-style-type: none"> <li>→ Immune priming</li> <li>→ HIF-1<math>\alpha</math>-mediated memory</li> </ul>	<b>Hif-1<math>\alpha</math><sup>-/-</sup></b> <ul style="list-style-type: none"> <li>→ Defects in motility, aggregation and bactericidal activity</li> <li>→ Protection against LPS-induced sepsis</li> </ul> <b>Hypoxia and <i>Leishmania amazonensis</i> infection</b> <ul style="list-style-type: none"> <li>→ Improved infection control</li> </ul> <b>PHD2 haplodeficiency</b> <ul style="list-style-type: none"> <li>→ Angiogenesis → Prevention of ischemia</li> </ul> <b>PNS regeneration/ischemia</b> <ul style="list-style-type: none"> <li>→ Hif-1<math>\alpha</math> increase in macrophages → Macrophage induced angiogenesis</li> </ul> <b>Tumour-associated macrophages (TAMs)</b> <ul style="list-style-type: none"> <li>→ M2 skewed → Tumour growth and angiogenesis</li> </ul>	

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**Figure 3. A table summarising the metabolic phenotypes and physiological outcomes documented to date linking hypoxia to myeloid cell metabolism.**

The metabolic changes associated with elevated Hif-1 $\alpha$  and Hif-2 $\alpha$  expression are shown for neutrophils (left, orange) and macrophages/DCs (right, purple). The inactivation of both PHD2 and HIF-1 $\alpha$  protein is reported to result in dysregulated neutrophil and macrophage function in murine models.

in the macrophages in this model, and ROS was proposed as the mechanism of the parasite killing. In another study investigating the effect of macrophages on peripheral nervous system (PNS) regeneration, macrophages were shown to respond to hypoxia by migrating to the wound and inducing angiogenesis at the severed region [101]. In tumour models, macrophages can constitute up to 50% of the tumour mass [102]. They accumulate in poorly vascularised and hypoxic environments and are usually associated with a poor prognosis. Tumour-associated macrophages (TAMs) are thought to function by promoting tumour cell proliferation and angiogenesis [103]. Although TAMs are M2-skewed, whether they have the same metabolic phenotype of an M2 macrophage remains unknown. A summary diagram of the metabolic changes during hypoxic exposure and activation of immune cells and the inflammatory outcomes is shown in Fig 3.

### Immune memory and hypoxia

Recently, there have been numerous reports describing transcriptional and epigenetic changes within immune cells and linking these to the inflammatory response. The phenomenon termed “innate immune memory” has been demonstrated in monocytes, macrophages, natural killer cells and neutrophils [104]. In a study by Netea *et al*, the metabolite mevalonate was shown to mediate long-term reprogramming in monocytes in response to  $\beta$ -glucan via the activation of IGF1 receptor and mTOR subsequently resulting in histone modifications [105]. Multiple major metabolic pathways including glucose metabolism, glutaminolysis and the cholesterol synthesis pathway are upregulated in  $\beta$ -glucan-trained macrophages [106,107]. HIF is central to  $\beta$ -glucan training, and mice lacking myeloid-specific HIF-1 $\alpha$  lack the ability to induce trained immune responses to sepsis [106].  $\beta$ -glucan is a component of fungal cell walls and is associated with stable changes in histone trimethylation at H3K4, which confer protection from secondary lethal infection through priming of the production of pro-inflammatory cytokines [108]. A second metabolite known to act as an immunometabolite is 2-hydroxyglutarate. This metabolite is known to accumulate in cancer cells with gain-of-function mutations of the isocitrate dehydrogenase 1/2 genes [109]. *In vitro*, 2-hydroxyglutarate levels increase in response to hypoxia and T-cell receptor triggering through a hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ )-dependent mechanism [21]. The authors of this study proposed that 2-hydroxyglutarate functions by inhibiting 2-oxoglutarate-dependent dioxygenases that demethylate histones or oxidise 5-methylcytosine in DNA as 2-hydroxyglutarate treatment resulted in higher methylation in CD8<sup>+</sup> T cells. In a second study, Burr *et al* [110] showed that 2-hydroxyglutarate inhibits both HIF $\alpha$  prolyl hydroxylases (PHDs) and TET 2-oxoglutarate-dependent dioxygenases. Conversely, hypoxic preconditioning has also been shown to result in innate immune memory in a HIF-1 $\alpha$ -dependent manner. Exposure to prolonged hypoxia prior to infection protects against the increase in morbidity and mortality that is observed with acute hypoxia [111]. These changes are mediated, in part, by neutrophils and associated with reduced HIF-1 pathway and metabolic gene expression. It is not known whether the neutrophil-driven memory effects are driven by immunometabolites. Further work is required in order to

#### Box 1: In need of answers

- How do innate immune cells sense metabolic states?
- What are the consequences of different metabolic environments on innate immune cell function and survival? Is innate immune metabolic reprogramming context dependent?
- Can our understanding of metabolic reprogramming in innate immune cells offer a potential therapeutic opportunity for the treatment of inflammatory diseases?

determine the role of immunometabolites in regulating innate immune cell memory (see also Box 1).

### Concluding remarks

Hypoxia is a prominent feature of inflammation associated with numerous disease states. Immune cells at hypoxic sites are required to maintain functional diversity and plasticity, as initially they have to adopt a pro-inflammatory phenotype and then later switch to a pro-resolution and repair phase. The regulation of immune metabolism by the HIF/hydroxylase pathway provides one mechanism by which innate immune cells can adapt to the hypoxic tissue environment. The immunological niche itself is likely to impact further on these metabolic adaptations, with evidence that small metabolites can in addition to their classical function act as signalling molecules regulating multiple cellular functions. This implicates impaired metabolism with impaired innate cell function. A better understanding of the metabolic states and sensing at the different inflammatory phases and the crosstalk with the HIF signalling pathway will be key to developing future therapeutic drugs for the treatment of diseases involving immune cells.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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