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Editorial

Editorial: **A PHD in macrophage survival**

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Tissue hypoxia is a frequently encountered condition that occurs when the cellular demand for molecular oxygen exceeds the vascular supply. There is an absolute requirement of oxidative metabolism for molecular oxygen that acts as the final electron acceptor of the mitochondrial electron transport chain. Therefore, hypoxic exposure represents a severe and acute threat to bioenergetic homeostasis and consequently, to cell, tissue, and ultimately organism survival. With this in mind, it is perhaps not surprising that over the course of evolution, metazoans (multicellular animals) have developed mechanisms by which to adapt to hypoxia through the induction of genes that promote an adaptive response, which increases cell, tissue, and organism survival during a period of oxygen deprivation [1, 2].

Our understanding of the mechanism(s) underpinning the cellular response to hypoxia was revolutionized by the discovery of HIF, a highly conserved, oxygen-sensitive transcriptional regulator of the adaptive response to hypoxia [3]. This was followed, almost a decade later, by the identification of HIF hydroxylases as the key oxygen sensors that confer hypoxic sensitivity upon this transcription factor [4, 5]. Under conditions of normoxia (where oxygen supply exceeds demand), most of the oxygen entering a cell is used for the generation of ATP through mitochondrial oxidative metabolism; however, there is usually some "non-mitochondrial" oxygen available that can be used for alternative purposes, such as the regulation of HIF.

The HIF-PHDs (PHD1, PHD2, and PHD3, also known as EGLN2, EGLN1, and EGLN3, respectively) are a family of dioxygenases that use this non-mitochondrial molecular oxygen as a co-substrate in the hydroxylation of two residues, in what is termed the oxygen-dependent degradation domain of the HIFα isoform (Pro 402 and Pro 564 on HIF-1α). When hydroxylated on these residues, the HIFα subunit is a target for the von Hipple Lindau protein, which recruits an E3 ubiquitin ligase complex that ubiquitinates HIF, leading to its proteasomal degradation. This process is prevented in hypoxia, leading to the rapid stabilization of HIFα, which is then free to translocate to the nucleus, bind to HIF1β/aryl hydrocarbon receptor nuclear translocator, and form the transcriptionally active HIF complex. There also exists a HIF2 homolog that regulates an overlapping but distinct subset of hypoxia-inducible genes, and genes regulated by HIF orchestrate the cellular adaptive response to hypoxia and include those that regulate the angiogenic (e.g., vascular endothelial growth factor), erythropoietic (e.g., erythropoietin), and metabolic (e.g., glycolytic enzymes) response to hypoxia. Therefore, hydroxylases are key oxygen sensors expressed in all cells that regulate the adaptive response to hypoxia and promote a return to oxygen homeostasis.

Whereas hypoxia has long been known to be associated with growing tumors and ischemic tissues, where blood flow is clearly disrupted or diminished, it has recently become appreciated that hypoxia is also a key feature of chronically inflamed tissues, such as inflamed intestinal tissue in IBD or inflamed joints in rheumatoid arthritis. The reasons for the co-occurrence of hypoxia and inflammation are likely a result of a dysfunctional vasculature as a result of chronic inflammation and associated fibrosis and increased oxygen consumption in highly metabolically active, inflamed tissues. A recent study has demonstrated that the neutrophilic oxidative burst associated with neutrophil infiltration into inflamed tissues drives a large increase in oxygen consumption, which is sufficient to be a major driver of hypoxia in inflamed tissues [6]. Therefore, hypoxia is an important microenvironmental feature of inflamed tissues. However, as well as being a consequence of inflammation, hypoxia also impacts significantly on its progression through the regulation of immune cell function via HIF [7]. Therefore, the relationship between hypoxia and inflammation is multifactorial and complex. Against this background, it is difficult to predict the net impact of activating hypoxia-sensitive pathways upon the progression of inflammation in vivo. In addressing this question, recent studies have demonstrated that mimicking hypoxia using pharmacologic hydroxylase inhibition is

Abbreviations: Angptl2=angiopoietin-like protein 2, EGLN=Egl nine homolog, HIF=hypoxia-inducible factor, IBD=inflammatory bowel disease, KO=knockout, PHD=prolyl hydroxylase

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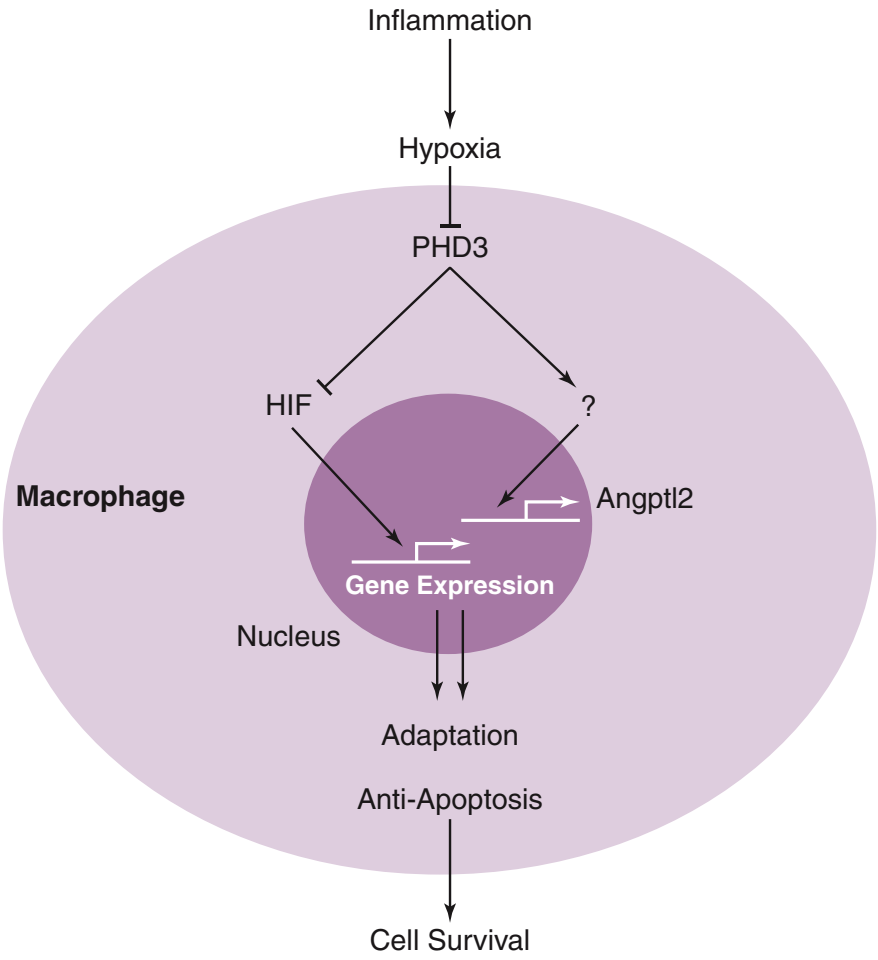


Figure 1. Chronically inflamed tissues become hypoxic as a result of reduced oxygen delivery and increased oxygen consumption at the inflamed site. Hypoxia-dependent PHD3 inhibition in macrophages promotes cell survival through the activation of HIF-dependent adaptive pathways and HIF-independent antiapoptotic pathways, including decreased expression of Angptl2.

protective in a range of models of inflammatory conditions, including colitis, ischemia/reperfusion, and sepsis [7]. This indicates that at least in these conditions, the net effect of mimicking hypoxia through pharmacologic hydroxylase inhibition is anti-inflammatory. Because of the potential importance of this in the development of new, anti-inflammatory agents, significant attention is now being focused, elucidating the links between hypoxia-sensitive pathways and the regulation of inflammation and understanding the mechanism(s) of anti-inflammatory action of hydroxylase inhibitors.

The PHD3 isoform is induced in cells in hypoxia in a HIF-dependent manner and has been proposed to play a role in a negative-feedback loop, curtailing the HIF-dependent response in prolonged hypoxia (presumably, to prevent excessive angiogenesis and other adaptive processes). Interestingly, neurons derived from a homozygous PHD3 KO mouse demonstrate decreased rates of apoptosis with a subsequent disruption of sympathoadrenal functions, including the regulation of blood pressure [8]. Furthermore, PHD3 KO mice demonstrate an enhanced innate immune response in a model of abdominal sepsis [9].

In this issue of *JLB*, Swain et al. [10] have investigated further the impact of altered PHD3 expression/activity on macrophage function. In their study, they provide convincing evidence that in macrophages, PHD3 plays a key role in the regulation of apoptosis. They demonstrate further that macrophages deficient in PHD3 have decreased levels of stress-induced apoptosis. Interestingly, the antiapoptotic effects of PHD3 KO are independent of alterations in HIF and instead, appear to occur via reduced expression of Angptl2, an extracellular protein that is structurally similar to angiopoietins but has not been associated previously with regulation by hypoxia [11]. These data provide evidence that as well as promoting an adaptive response to hypoxia through HIF in many cell types, PHD3 KO or inhibition can also promote cell survival in macrophages that may be associated with providing a transient survival signal during a period of hypoxic stress (**Fig. 1**). This may have significant implications for macrophage survival in vivo, as these cells migrate from the well-oxygenated bloodstream to sites of hypoxic inflammation, where they carry out their main immune function.

Whereas this work further enhances our understanding of the complex crosstalk that exists between inflammatory and hypoxic signaling pathways, some key questions remain to be answered. Primarily, the identity of the key factors regulating Angptl2 in macrophages, where PHD3 is inhibited, remains unknown. As this regulation occurs at the mRNA level, presumably, it is a result of a PHD3-sensitive transcription factor. It is intriguing that the authors have identified that this is not HIF, raising the possibility of an independent hydroxylase/hypoxia-dependent transcription factor. This is consistent with emerging evidence that hydroxylases play key roles in cell signaling outside of the HIF pathway.

Of key importance is how this work contributes to the potential use of pharmacologic hydroxylase inhibitors in the treatment of inflammatory disorders. Inflammatory disease represents a major burden on society, and conditions, such as IBDs, rheumatoid arthritis, and psoriasis, are still in need of new and improved therapeutics. Furthermore, inflammation is a major predisposing risk factor for cancer development (e.g., ~30% of patients suffering from chronic IBD will develop colon cancer). Therefore, the development of our un-

derstanding of the impact of microenvironmental hypoxia in the context of chronic inflammation will open new windows of therapeutic potential in a wide range of inflammatory disease states.

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Editorial: **The contribution of myeloid-derived suppression to inflammatory disease**

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Both innate and adaptive immune mechanisms exist to restrain inflammatory T lymphocyte responses and protect against excessive host damage. Aberrant activation of regulatory mechanisms, however, can lead to strong immunosuppression that is detrimental in infectious diseases and cancer. In the adaptive immune response, T_{regs} represent one such regulatory mechanism with well-defined markers and roles in restricting inflammation [1]. The characteristics exhibited by immunosuppressive cells of the innate immune response are not as clear. It has been known for a number of years that a heterogeneous population of $CD11b^+Gr1^+$ cells, collectively known as MDSCs, is associated with the repression of anti-tumor T cells. Likewise, MDSC-like cells are also expanded under inflammatory conditions, which may represent a relatively unexplored regulatory mechanism with the potential to influence the course and outcome of the immune response.

In mice, MDSCs can be generally classified as G-MDSCs ($CD11b^+Ly6G^+$) or M-MDSCs ($CD11b^+Ly6C^+$), with a variety of additional markers and suppressor mechanisms further subdividing the population (reviewed in ref. [2]). Both types of MDSC subsets are up-regulated in various cancers, where they have been shown to inhibit deleteriously T cell-dependent tumor immunity [2]. Although human and mouse MDSCs can use many different suppressive mechanisms, the predominant means are arginase 1 and iNOS enzymatic activity, which are important for L-arginine starvation and the inhibition of T cell signaling [2, 3]. Additionally, MDSCs have been implicated in inhibiting the activation of anti-tumorigenic cells, such as $CD8^+$ T cells and NK cells, while still using various cell-contact and soluble mechanisms to promote reciprocally antigen-specific activation and ex-

Abbreviations: cLP=colon lamina propria, DSS=dextran sulfate sodium, EAE=experimental autoimmune encephalomyelitis, G-MDSC=granulocytic myeloid-derived suppressor cell, IBD=inflammatory bowel disease, M-MDSC=monocytic myeloid-derived suppressor cell, MDSC=myeloid-derived suppressor cell, MLN=mesenteric lymph node, T_{reg}=regulatory $CD4^+$ T cell

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