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Hypoxia and hypoxia-inducible factors as regulators of T cell development, differentiation, and function

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Abstract Oxygen is a molecule that is central to cellular respiration and viability, yet there are multiple physiologic and pathological contexts in which cells experience conditions of insufficient oxygen availability, a state known as hypoxia. Given the metabolic challenges of a low oxygen environment, hypoxia elicits a range of adaptive responses at the cellular, tissue, and systemic level to promote continued survival and function. Within this context, T lymphocytes are a highly migratory cell type of the adaptive immune system that frequently encounters a wide range of oxygen tensions in both health and disease. It is now clear that oxygen availability regulates T cell differentiation and function, a response orchestrated in large part by the hypoxia-inducible factor transcription factors. Here, we discuss the physiologic scope of hypoxia and hypoxic signaling, the contribution of these pathways in regulating T cell biology, and current gaps in our understanding. Finally, we discuss how emerging therapies that modulate the hypoxic response may offer new modalities to alter T cell function and the outcome of acute and chronic pathologies.

Keywords T cell · Oxygen · Hypoxia · Hypoxia-inducible factor · Metabolism

Abbreviations

EPO	Erythyropoietin
FoxP3	Forkhead box P3
HIF	Hypoxia inducible factor
HRE	Hypoxia response element
IBD	Inflammatory bowel disease
IEL	Intraepithelial lymphocyte
kPa	Kilopascal
mm Hg	Millimeter of mercury
PHD	Prolyl hydroxylase
TCR	T cell receptor
Tregs	Regulatory T cells
VEGF	Vascular endothelial growth factor
VHL	von Hippel-Lindau

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Oxygen supply and consumption

Oxygen is central to life for multicellular, eukaryotic organisms and is required for aerobic respiration and robust mitochondrial generation of ATP [1, 2]. In mammals, oxygen distribution is critically regulated by the vascular system, which continually traffics oxygen-saturated red blood cells throughout the body. Despite this robust system, oxygen distribution in the body is not always uniform, with various cells and tissues transiently experiencing periods of reduced oxygen availability, a state referred to as hypoxia. Given the essential requirement for oxygen in many cellular processes, and the lethal effects of oxygen deprivation at both the tissue and organismal level, metazoans have developed a tightly regulated response that

allows for adaptation to both acute and chronic hypoxia. Although different tissues and cells have distinct thresholds and susceptibility to hypoxia [3], at a cellular level, hypoxia and hypoxic responses generally occur at a $PO_2 \leq \sim 1$ kPa ($\leq \sim 7$–10 mm Hg or ~ 1 % O_2) [4].

In order to understand hypoxia in both physiologic and disease states, it is important to gain an appreciation for the basics of oxygen uptake and delivery, and the surprisingly steep gradient of PO_2 when comparing atmospheric PO_2 relative to in situ PO_2. At sea level, the total atmospheric pressure is 101 kilopascals (kPa) (760 mm Hg), with 21 % of this atmosphere comprised of oxygen, resulting in an atmospheric partial pressure of oxygen (PO_2) of 21.2 kPa (159 mm Hg) [3]. Following inspiration of oxygen with each breath, red blood cells rapidly absorb oxygen resulting in a maximal O_2 tension in the pulmonary vasculature of 13 kPa ($\sim$98 mm Hg) [3]. As oxygen is delivered to tissue, venous blood PO_2 decreases to 5.3 kPa ($\sim$40 mm Hg). Notably, within interstitial spaces of tissue, the average PO_2 is 2.7–5.3 kPa ($\sim$20–40 mm Hg) with intracellular oxygen tensions ranging from 1.3 to 2.7 kPa ($\sim$10–20 mm Hg). These intracellular oxygen tensions are only a fraction of the PO_2 present in normal atmospheric air and are close to the threshold at which hypoxia and hypoxic responses generally occur at a $PO_2 \leq \sim 1$ kPa ($\leq \sim 7$–10 mm Hg or ~ 1 % O_2) [4].

Beyond these average baseline values, the O_2 tension a particular cell experiences is dynamically regulated by metabolic supply and demand in the tissue and within the cell itself. Factors that regulate this include: (i) circulatory distance from lung oxygenation, (ii) relative proximity of cell(s) to the vasculature, (iii) rate of local oxygen consumption, and (iv) rate of oxygen consumption within the cell. For example, a quiescent cell has very low metabolic demands, in contrast to a highly proliferating cell (such as an activated lymphocyte) [5]. Additionally, certain cells (e.g., a neutrophil) have a pronounced ability to consume oxygen via NADPH oxidase generation of reactive oxygen species [6], which in itself can alter oxygen levels in surrounding tissues and affect cellular responses.

Hypoxia occurs in a variety of physiologic and pathologic contexts

Physiologic states of hypoxia

Hypoxia is defined as a state of reduced or inadequate oxygen availability. Though distinct tissues and cells have varying degrees of susceptibility to hypoxia [3], at a cellular level, hypoxia and hypoxic responses generally occur at a $PO_2 \leq \sim 1$ kPa ($\leq \sim 7$–10 mm Hg or ~ 1 % O_2) [4]. While cells directly in the bloodstream are unlikely to experience hypoxia except under severe pathologies, it is noteworthy that many tissues experience low PO_2 levels, even under normal physiologic conditions. This has been demonstrated through the use of oxygen-sensing probes in living, anesthetized animals, where investigators found that the mean PO_2 in the thymus was 1.3 kPa (10 mm Hg), with the majority of readings between 0 and 2.3 kPa (0–17 mm Hg) [7]. Moreover, microelectrode measurements of PO_2 tensions in the spleen ranged from 0.5 to 4.5 kPa (4–34 mm Hg), depending on the relative proximity to the splenic artery [8]. Further evidence for hypoxic microenvironments has been possible through the use of nitroimidazole-based compounds that are specifically reduced within cells experiencing low oxygen tension (generally PO_2 <1.3 kPa or <10 mm Hg). In low oxygen tension, these compounds generate reactive nitrogen intermediates that form protein adducts that can be recognized by specific antibodies [6, 9] and thereby identify cells with low oxygen tension. This method has permitted demonstration that T cells are present in a hypoxic environment following sepsis [10], that the bone marrow contains hypoxic niches that are home to hematopoietic stem cells [11], and that intestinal inflammation is characterized by inflammation-associated hypoxia [12].

While a full discussion of tissues that experience conditions of "physiologic hypoxia" (here defined as in situ hypoxia in the resting, healthy individual) is beyond the scope of this article, the presence of hypoxic microenvironments has been observed in a range of tissues, including the retina, the medulla of the kidney, the epidermis of the skin [13], the thymus [7, 11], hypoxic niches within the bone marrow [11, 14], and even regions within the spleen [8]. Moreover, given that the lumen of the gastrointestinal (GI) tract is essentially anoxic (i.e., containing no oxygen), the epithelium lining of the GI tract also demonstrates steady state hypoxia [6].

Beyond these homeostatic/physiologic areas of hypoxia, inflammatory sites can also be characterized as hypoxic [6]. Inflammation-associated hypoxia (or inflammatory hypoxia) can result from a combination of factors that result in a shift in metabolic supply and demand. First, within an inflamed tissue, there is an accumulation of metabolically active leukocytes. For example, in the case of intestinal inflammation during chemically induced colitis, neutrophilic infiltration into the colon and significant oxygen consumption due to generation of the respiratory burst can lead to depletion of local oxygen [6]. Additionally, during inflammation, the generation of interstitial edema can increase the intercapillary distance [3], further impairing oxygen transport to cells at a site of inflammation. Oxygen consumption also increases with elevated temperature, which is particularly relevant in the context of inflammation-associated fever [3]. All of these factors likely contribute to the development of a hypoxic environment at inflamed sites,

Table 1 Examples of pathologic conditions characterized by hypoxia

Pathologic condition	Possible causes of hypoxia	Duration of hypoxia
Ischemia/reperfusion (e.g., stroke, myocardial infarction, hepatic ischemia, renal ischemia)	Acute vessel obstruction [21]	Acute (seconds–minutes)
Transplantation	Transient disruption of tissue perfusion, vasculature [15]	Acute (minutes–hours)
Wound healing	Metabolic activity of cells at site [18]	Acute (hours–days)
Cancer (solid tumors)	Insufficient and irregular vascularization to solid tumor [20]	Acute to chronic
Vascular disease (e.g., sickle cell disease)	Impaired red blood cell trafficking and oxygen delivery [21]	Acute to chronic
Obstructive sleep apnea	Impaired breathing during sleep [17]	Intermittent (over hours)
Chronic infection (e.g., granulomas)	Metabolic activity of cells at site, tissue necrosis [16]	Chronic (days–months)
Obesity	Insufficient vascularization, Insufficient oxygen diffusion due to cell size [22]	Chronic (weeks–months)

References are indicated in "Possible causes of hypoxia" column

which can modulate the responses of infiltrating cells, including T lymphocytes.

Pathologic states of hypoxia

At present, hypoxia has been identified as an important cofactor in a wide range of pathologic states (reviewed in [2, 15–18]; Table 1). Prominent examples of diseases in which hypoxia is thought to have significant impact include: (i) solid tumors, with an insufficient or irregular vascular bed supply [19, 20], (ii) ischemic injury, including liver or kidney ischemic injury, myocardial infarction, and transplants [21], and (iii) obesity, in which adipose tissue hypoxia contributes to metabolic and inflammatory changes [22]. In each of these contexts, T cells are thought to have the capacity to influence the nature of the disease process [23–30]. However, the specific contribution of hypoxic signaling within T cells in these disease processes remains poorly characterized.

Oxygen tension in T cell environments

T cells frequently encounter a wide range of oxygen tensions given their highly mobile nature (Fig. 1; Table 2). During development, thymocytes reside within the relatively low oxygen environment of the thymus [7], whereas mature T cells circulating in the blood will experience a relatively high oxygen environment. However, depending upon the specific localization within the spleen, some mature T cells can also be found in hypoxic areas even under homeostatic conditions. Furthermore, T cells present in the lymphatic system experience a range of oxygen tensions ($\sim$1.1–4.7 kPa or 8–35 mm Hg), the lower end of which is sufficiently low to induce hypoxic responses, including Hif-1α protein stabilization [31–33]). Most certainly, effector T cells present at sites of inflammation or retained within non-lymphoid tissues have a high probability of prolonged exposure to a hypoxic environment. For example, in the context of human chronic inflammatory diseases, T cells present in inflammatory lesions in inflammatory bowel disease (Crohn's disease and ulcerative colitis) [34] and rheumatoid arthritis [35] express Hif-1α. This evidence suggests that T cell responses in the context of inflammation are likely influenced by both hypoxia exposure and modulation of HIF expression.

Oxygen-sensing machinery and the response to hypoxia

Following exposure to inadequate oxygen levels, hypoxia triggers a multi-pronged adaptive response. For example, exposure to high altitude and the decreased PO_2 resulting from reduced atmospheric pressure elicits an immediate increase in respiratory rate and an increase in erythropoietin (EPO) that stimulates the production of red blood cells; these adaptations function to enhance the oxygen-carrying capacity of the blood [36]. At a cellular level, hypoxia induces changes in the mRNA expression of hundreds of genes, including those involved in metabolic pathways as well as tissue adaptive responses (e.g., increased production of vascular endothelial growth factor, VEGF) [37, 38].

Hif-1α is a master regulator of the hypoxic response

To date, the best-characterized transcriptional effector of the hypoxic response is Hif-1α. Hif-1α is a basic helix-loop-helix, PAS domain containing transcription factor that is profoundly induced by hypoxia. In hypoxia, Hif-1α heterodimerizes with the constitutively expressed Hif-1β

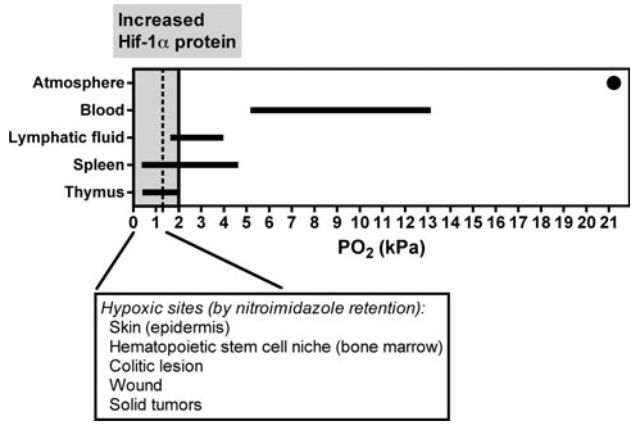

Fig. 1 Physiologic oxygen tensions in situ are significantly lower than atmospheric oxygen tension, with multiple tissues experiencing "physiologic hypoxia" during normal inspiration of atmospheric air. Graph depicts the range of oxygen tensions in multiple tissues (indicated at *left*), with published ranges of oxygen tensions indicated by the *black bar* on the plot. The *gray shaded* area, between 0 and 2 kPa, indicates the PO_2 range at which Hif-1α protein has increased stability, a marker of the hypoxic response. The *vertical dashed line* indicates a PO_2 of 1.3 kPa, the PO_2 at which cells generally retain nitroimidazole compounds, indicating cells or tissues experiencing hypoxia. Additional examples of tissues that stain positive with nitroimidazole compounds are indicated at the *bottom* of the figure. Please see text for additional details and references

(also known as Arnt, the aryl hydrocarbon receptor nuclear transporter) and translocates to the nucleus, where it binds to hypoxia response elements (HREs) in the promoters of a variety of genes [38, 39]. HIF transcriptional targets include glycolytic machinery (e.g., Glut1, Pgk1), VEGF, and the chemokine receptor CXCR4. While some Hif-1α targets are conserved across multiple cell types, Hif-1α is also clearly capable of mediating cell type-specific transcriptional responses [39].

Hif-1α is regulated at multiple stages, including transcriptional, translational, and post-translational levels [40]. The two best described oxygen-dependent processes that influence Hif-1α stability and function are as follows:

(i) Hif-1α is hydroxylated on two highly conserved proline residues by prolyl hydroxylase enzymes (PHD1-3), which in turn allows the von Hippel–Lindau protein, an E3 ubiquitin ligase, to bind, ubiquitinate, and target Hif-1α for proteasomal degradation [41–43]. Notably, prolyl hydroxylation and PHD activity are regulated by molecular oxygen, such that in conditions of limited oxygen availability, PHD function is inhibited, disrupting this proteolytic cascade [44].

(ii) Hif-1α is also hydroxylated in an oxygen-dependent reaction on a highly conserved asparagine residue by FIH-1 (Factor inhibiting HIF-1), which blocks the ability of Hif-1α to recruit transcriptional coactivators such as p300/CBP, in turn limiting transcriptional activation by Hif-1α under non-hypoxic conditions [45, 46]. Similar to the PHDs, in conditions of limited oxygen, FIH-1 function is inhibited, disrupting hydroxylation of this asparagine residue and allowing for enhanced HIF transcriptional activity [44].

Based on these mechanisms, in normoxia, Hif-1α is rapidly degraded and has limited transcriptional activation capacity. In contrast, in limiting oxygen conditions, Hif-1α stability and activity are substantially increased, allowing a transcriptional response that is tightly induced in hypoxic conditions.

Table 2 Physiologic sites where T cells may encounter low oxygen tension

Anatomical site	T cell subset
Thymus	All thymocytes
Intestine	Intraepithelial lymphocytes (IELs)
Skin	γδ T cells
Wounds	γδ T cells
Sites of inflammation	Various
Bone marrow	CD8 memory T cells

Molecular regulators of the hypoxic response beyond Hif-1α

Hif-2α was first identified as endothelial PAS domain containing protein 1 (EPAS1), a PAS domain containing transcription factor with 48 % identity to Hif-1α, hypoxia-inducible expression, and HRE-binding capacity [47]. Hif-2α is an important transcriptional regulator of hypoxic responses, controlling a variety of processes including hypoxic regulation of EPO synthesis [48, 49] and macrophage function [50, 51]. The interplay between Hif-1α and Hif-2α in hypoxic gene regulation remains an evolving field. It is worth noting, however, that in different contexts, these transcriptional regulators can have redundant, unique, or antagonistic functions [52].

Beyond the HIFs, hypoxia triggers multiple transcriptional cascades (reviewed in [53]). For example, hypoxia activates the NF-kB pathway through p65 and p50 stabilization and nuclear translocation that induces transcription of many genes [54]. Interestingly, NF-kB, which is a master regulator of inflammatory responses, is essential for the induction of baseline Hif-1α mRNA [55], demonstrating that hypoxia, Hif-1α, and NF-kB are intimately linked in regulating both hypoxia and inflammation. Interestingly, in T cells, stimuli that activate NF-kB such as antigen or cytokine stimulation also induce Hif-1α protein (see below for more details).

Recent studies have further revealed additional molecular regulators of the hypoxic response. These include:

1. hypoxic-elicited microRNAs, capable of mediating regulatory changes at the transcriptional and post-transcriptional level [56],
2. epigenetic modifications, mediated in part by hypoxic induction of enzymes involved in DNA methylation and histone acetylation. These changes are capable of allowing new genes to be expressed in hypoxia, as well as to repress expression of additional targets [57],
3. the production of reactive oxygen species, resulting from perturbations in mitochondrial respiration, which at least in certain contexts is required for the induction of Hif-1α and the cellular hypoxic response [58–60].

The relative contribution of these various effector mechanisms into the composite effect of hypoxia remains an area of intense investigation, but is highly likely to depend on particular cell type and differentiation status.

Alternative inducers of HIFs

Although hypoxia is a primary inducer of HIF protein stabilization, non-hypoxic stimuli that disrupt the PHD/VHL/HIF proteolytic cascade can also induce accumulation of HIF. The PHD proteins require multiple cofactors for Hif-1α prolyl hydroxylation: molecular oxygen, 2-oxoglutarate (also known as alpha-ketoglutarate), iron(II) and vitamin C or glutathione [44]. In the case of hypoxia, molecular oxygen becomes limiting, resulting in impaired prolyl hydroxylation and the accumulation of Hif-1α protein. Under normoxic conditions, alternate mechanisms of HIF stabilization can stabilize HIF by interfering with cofactors required for the PHD prolyl hydroxylation reaction [61]. Since iron(II) is required for PHD function, HIF stabilization can be induced by iron chelators including desferrioxamine and bacterial siderophores [62, 63]. In addition, cobalt chloride is an effective stabilizer of Hif-1α and can induce hypoxic responses in vivo [64]. Finally, PHD activity is regulated by metabolites within the cell, and elevated levels of succinate or fumarate can inhibit PHD activity [65, 66], demonstrating that PHD enzymes may serve as a metabolic sensor beyond the measurement of molecular oxygen [67, 68].

Hif-1α stabilization in T cells

Hif-1α protein stabilization can occur in multiple contexts in T cells (Fig. 2). While hypoxic exposure of T cells results in modest Hif-1α stabilization [35], there are at least two additional, non-conventional ways in which T cells induce Hif-1α protein. First, T cell receptor (TCR) stimulation results in robust Hif-1α protein stabilization that can be further enhanced by combining TCR stimulation with hypoxia [35, 69]. This increased Hif-1α protein synthesis is mediated by the PI3 kinase/mTOR pathway [69]. TCR activation can also induce expression of distinct splice isoforms of Hif-1α mRNA in human and mouse T cells, although the unique contribution of these splice isoforms to gene regulation remains unclear [8, 70]. A second mechanism of Hif-1α induction in T cells is mediated by culture with the pro-inflammatory cytokine IL-6, which activates the STAT3 transcription factor [71]. Mechanistically, it is unknown how this IL-6-driven Hif-1α expression integrates with the normal proteolytic machinery that restricts Hif-1α protein stability. In addition, it is currently unknown whether Hif-1α stabilization after TCR stimulation results strictly due to the pathways mentioned above, or whether intracellular oxygen consumption following TCR activation may also contribute to Hif-1α protein expression within activated T cells.

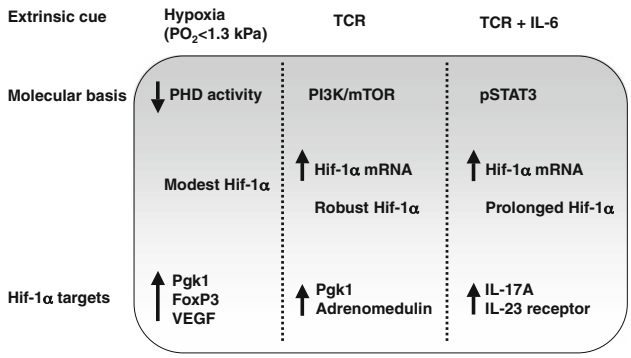

Fig. 2 Hif-1α protein expression within T cells can be induced by multiple mechanisms, with distinct outcomes on magnitude and duration of Hif-1α expression. Of the three extrinsic cues known to induce Hif-1α protein (listed in the *top row* of the figure), each uses a distinct mechanism of induction. Examples of identified Hif-1α target genes for each pathway are included

How oxygen availability influences T cell function

While most in vitro assays for T cell function are performed at normal atmospheric oxygen (~21 % O_2, ~21.2 kPa PO_2, or ~159 mm Hg), these concentrations are dramatically higher than even the most oxygen-rich environment in vivo [3]. Significantly, when T cells are activated in conditions of physiologic oxygen tension (2–5 % O_2, ~2–5 kPa, or ~15–38 mm Hg), there are a number of changes in T cell dynamics. First, T cells activated at physiologic O_2 tension have reduced proliferation and expansion following TCR stimulation. Moreover, T cells shift their metabolism to glycolysis and have an altered redox state that is more closely aligned with that observed in vivo [72–75]. Culture in physiologic O_2 levels also alters the acquisition of CTL functions, resulting in diminished cytokine production (IL-2,

IFN-γ), yet increased VEGF production, and enhanced lytic capacity in CD8 T cells [8].

The observation of reduced proliferation under hypoxia is not unique to T cells. Indeed, the anti-proliferative effect of hypoxia has long been appreciated, with proposed mechanisms for this phenomenon ranging from upregulation of cell cycle inhibitor proteins [76], to recent data demonstrating that Hif-1α can directly interact with Mcm replication proteins to regulate cell cycle progression [77]. In the context of T cells, however, the molecular basis of this reduced proliferation remains unknown, but can occur even in the context of strong TCR signals, CD28-mediated costimulation, and addition of exogenous IL-2 [128]. Despite these strong mitogenic conditions, reduced proliferation may be influenced by hypoxia-mediated alterations to TCR signaling in activated T cells (e.g., impaired calcium flux due to hypoxia-mediated changes in the Kv1.3 potassium channel) [78].

In vitro T cell cultures in physiologic O_2 can also influence T cell survival, although the overall effect of hypoxia on apoptotic pathways in vitro is influenced by the timing and cell type analyzed. For example, human T cells activated in hypoxia are protected from activation-induced cell death, potentially through hypoxic transcriptional induction of adrenomedulin [79]. Conversely, culture of activated human T cells at physiologic O_2 can result in a significantly elevated rate of spontaneous apoptosis in certain T cell subsets [72].

In vitro oxygen availability also clearly influences cytokine production. Once again, the overall effect of hypoxia on cytokine production in vitro is influenced by the culture conditions and cell type analyzed [8, 80]. These studies frequently involve analysis of activated peripheral blood T cells, a caveat that makes it difficult to determine the specific contribution of hypoxia of specific T cell subsets. Notably, one recent study using carefully controlled preparations of naïve T cells demonstrated that culture at physiologic O_2 levels increases IL-17 production and Th17 differentiation [81], an intriguing observation particularly in light of the increasing genetic data that Hif-1α is required for optimal Th17 differentiation (see below for further discussion).

While the above studies clearly demonstrate the impact of oxygen availability on T cells, it is nonetheless clear that T cells can still be functional in a hypoxic environment. Indeed, T cells are able to preserve functionalities including cytokine production even in conditions of severe metabolite restriction including limited oxygen glucose [73].

Cell-extrinsic effects of hypoxia on T cells

Hypoxia can also affect T cells in a cell-extrinsic manner, whether the hypoxic effect occurs in stromal cells or other hematopoietic cell types [24, 82]. Some specific examples include:

1. Hypoxia can induce chemokines to selectively recruit T cell subsets. Recent work analyzing the link between cancer, hypoxia, and tumor-protective effects demonstrated that tumor-associated hypoxia induces the expression of chemokine CCL28, which selectively induces the recruitment of regulatory T cells (Tregs) leading to induction of tumor tolerance and neoangiogenesis [83].
2. Hypoxia can potently influence antigen-presenting cells by altering their antigen presentation and T cell stimulation capacity [82]. One emerging theme is that tumor hypoxia generates macrophages that can suppress T cell responses. This suppressive phenotype is mediated by hypoxic induction of arginase 1 and nitric oxide synthase, both that restrict T cell activation. Strikingly, induction of this hypoxic pathway in macrophages is required for optimal tumor growth [84, 85].
3. Hypoxia can alter target cell susceptibility to CD8 T cell-mediated cytotoxicity as evidenced by the increased resistance of hypoxic tumor cells to CTL-mediated lysis. This increased resistance to CD8 T cell-mediated killing is correlated with the hypoxic induction of tumor cell autophagy [86, 87].

Additional cofactors in the hypoxic microenvironment

It is noteworthy that in hypoxic microenvironments, there are often additional alterations in nutrients and metabolites, often driven by the hypoxic response, which can influence T cell function. Perhaps, the best example of this is the generation of extracellular adenosine and promotion of adenosinergic signaling as a negative feedback loop to restrict T cell activation (reviewed in [23]). Another frequent microenvironmental change with hypoxia is elevated lactate, resulting from glycolysis, that can decrease the local pH; there are emerging data that lactate can also influence T cell function [88, 89].

Hypoxia-inducible factors as intrinsic regulators of T cells

Hif-1α is essential for embryonic development and has widespread, cell type-specific roles in vivo [90]. Given this complexity, studies to address the role of Hif-1α in T cells have been advanced most significantly through the analysis of mice in which Hif-1α has been ablated specifically in T cells. For the remainder of this review, the focus will be on the intrinsic effects of Hif-1α on T cell responses. A

thorough discussion of the roles of HIFs in innate immunity has been recently reviewed elsewhere [82].

Hif-1α in thymocytes

To date, the genetic role of Hif-1α in thymocytes has been primarily characterized through gain-of-function studies. An early approach to analyze the role of HIFs in thymocytes was to specifically delete the von Hippel–Lindau (Vhl) E3 ubiquitin ligase in thymocytes, an approach that results in the stabilization of Hif-1α and Hif-2α [91]. Although Vhl targets multiple proteins for proteasomal degradation, HIFs are a major physiologic target [44]. Thymocyte deletion of Vhl results in a pronounced deficit in thymocyte development, with increased apoptosis of $CD4^{+}CD8^{+}$ double-positive thymocytes [91]. This phenotype is partially reversed following the deletion of Hif-1α on the Vhl-deficient background, indicating that constitutive Hif-1α expression in the absence of Vhl is deleterious to normal thymocyte development and differentiation, potentially by enhancing a caspase 8-dependent apoptotic pathway [91]. Constitutive activation of Hif-1α in Vhl-deficient thymocytes also resulted in a reduced calcium response following TCR cross-linking that resulted from increased removal of calcium from the cytoplasm via Hif-1α-induced increases in SERCA2 calcium pumps [92]. The physiologic role of the Hif-1α-regulated calcium response in thymocytes, however, remains unknown.

While these early studies provided important insights into the consequence of heightened Hif-1α activity within the thymus, an important gap in our understanding involves what effects Hif-1α deficiency alone has on thymocyte development (including effects on positive and negative selection).

Hif-1α as a negative regulator of T cell responses

Early genetic studies on the role of Hif-1α in T cells identified Hif-1α as a potential negative regulator of T cell responses, a concept pioneered by Michail Sitkovksy [10, 93]. The first studies on Hif-1α in T cells focused on in vitro models of T cell activation using Hif sufficient or deficient T cells from conditional Hif-1α KO mice, mice lacking the activation inducible Hif-1α isoform or bone marrow chimeras [93], and showed that Hif-1α-deficient T cells had enhanced production of cytokines including IFN-γ and IL-2. By using a model of cecal ligation and puncture, it was also determined that Hif-1α had an important role in negatively regulating T cell function in vivo [10]. Whereas wild-type mice had a high rate of mortality following cecal ligation, T cell Hif-1α-deficient mice had improved survival and decreased bacterial loads associated with enhanced cytokine production (including IL-2 and IFN-γ), increased proliferation, and enhanced NF-kB activity (both p50 and p65) in T cells [10].

Further evidence that Hif-1α may serve as an important negative regulator of T cells came from a study of vascular remodeling in mice lacking Hif-1α in T cells. Hif-1α-deficient T cells had enhanced production of IFN-γ and IL-2 following in vitro activation; interestingly, it was also observed that Hif-1α KO T cells produced less IL-17 (see below). T cell-specific Hif-1α KO mice also had increased inflammatory cell infiltration and vascular remodeling in a vascular injury model [94]. Additionally, in vitro activation of CD4 T cells from Hif-1α heterozygous mice demonstrated increased IFN-γ production and decreased eosinophilia in a model of antigen-elicited airway eosinophilia [80]. Consistent with the notion that Hif-1α can constrain T cell responses, studies by Ben-Shoshan et al. [95] demonstrated that hypoxia and Hif-1α can promote the transcriptional induction of FoxP3, to promote the generation of regulatory T cells (Tregs). Furthermore, our recent data demonstrate that hypoxia enhances the abundance of Tregs through direct transcriptional induction of FoxP3 and that Hif-1α plays a Treg-intrinsic role in promoting Treg function, to restrict intestinal inflammation [128].

While many of the above studies demonstrate that Hif-1α may constrain T cell responses, one important caveat is that the precise contribution of Hif-1α in CD4 versus CD8 T cells or in naïve versus effector T cells was often not thoroughly investigated. This latter point is particularly important given the differential functional capacities of naïve and effector T cells [96].

Hif-1α as a regulator of Treg versus Th17 differentiation

In contrast to the above studies showing that Hif-1α can serve as an important negative regulator of T cell responses, two recent reports identified an alternative role for Hif-1α in T cell differentiation in promoting the production of pro-inflammatory Th17 cells while simultaneously limiting the generation of Tregs [71, 97]. These conclusions were based on the observation that T cells lacking Hif-1α had impaired Th17 differentiation and increased production of Tregs. While the precise molecular basis for this observation differed between the two studies, both groups showed that Hif-1α was required for the induction of experimental autoimmune encephalitis, a Th17-driven murine model of autoimmunity.

The above genetic studies define a wide role for Hif-1α in T cells, with effects ranging from Hif-1α as a negative regulator of T cell responses to Hif-1α as a positive regulator of the highly pro-inflammatory Th17 subset (Table 3). How can Hif-1α have such diverse effects on T cells? While the molecular basis of Hif-1α regulation and targets

Table 3 Genetic analysis of hypoxic signaling in T cells

Gene (Gene symbol)	Knockout phenotype
Hif-1α (Hif1a)	Thymic deficit: Reduced cellularity, unknown mechanism [91]
	Peripheral T cells
	(i) Enhanced pro-inflammatory cytokines after in vitro TCR stimulation [93]
	(ii) Improved survival after sepsis [10]
	(iii) Increased inflammation after vascular injury [94]
	(iv) Impaired Treg function [128]
	(v) Defect in Th17 differentiation [71, 97]
Hif-1α-I.1	Enhanced pro-inflammatory cytokines after in vitro TCR stimulation [93]
Hif-2α (Epas1)	Unknown
Vhl (Vhl)	Thymic deficit: Increased apoptosis (Hif-1α dependent) [91]
	Thymocytes: Altered calcium signaling due to increased SERCA2 (Hif-1α dependent) [92]
PHD1 (Egln2)	Unknown
PHD2 (Egln1)	Unknown
PHD3 (Egln3)	Unknown
FIH1 (Hif1an)	Unknown

Note that loss-of-function alleles have been generated for all of these genes at an animal level. Of the indicated genes, deletion of Hif-1α, Hif-2α, Vhl, and PHD2 results in embryonic lethality and requires the use of floxed alleles for genetic analysis

in T cells remains an ongoing area of investigation, it is important to note:

1. Hif-1α can be induced by at least 3 distinct mechanisms in T cells (discussed above), with each condition inducing Hif-1α with a different magnitude and duration. At this time, it remains unclear what Hif-1α regulates in each of these contexts and how Hif-1α integrates with additional transcriptional pathways in T cells. For example, during Th17 differentiation, Hif-1α can be elicited by strong metabolic demands during IL-6 driven, STAT3-dependent Th17 differentiation [71, 97]. Conversely, Hif-1α induction in the context of hypoxia may occur in the absence of pro-inflammatory transcriptional pathways; in this later context, Hif-1α induction may be more important in hypoxia-driven Treg generation [95, 128].
2. In a broader sense, it is also important to note that T cell function is dynamically regulated by microenvironmental cues, and integrating Hif-1α into distinct T cell differentiation programs might be beneficial for multiple aspects of T cells (in both promoting and constraining inflammation). This concept of dual-purpose transcription factors has gained significant traction over the past three years, with an increasing list of transcription factors now shown to regulate both effector T cell function and regulatory T cell function, including IRF4, STAT3, T-bet, GATA3, Bcl-6, and Blimp-1 [98–105].

Based on this supposition, a future goal for this field of study will be to rigorously compare diverse models of in vitro and in vivo T cell function using Hif-1α-deficient models to gain a true understanding for whether Hif-1α constrains or promotes T cell inflammatory responses or whether the role of Hif-1α in modulating T cell responses is dependent on the T cell subset in which its expression is activated (e.g., naïve vs. effector T cell, Treg vs. Th17 cell).

Hif-1α in metabolic reprogramming of T cells

In recent years, there has been an increasing understanding of the central importance of metabolism in regulating T cell function [5, 106, 107]. Different subsets of T cells, as well as different stages following T cell activation, are characterized by the use of different metabolic pathways [97, 108, 109]. For example, upon T cell activation, T cells shift from a relatively metabolically quiescent state (characterized primarily by catabolic metabolism) to a highly metabolic phenotype (characterized primarily by anabolic metabolism and the induction of aerobic glycolysis) [106].

A major function of Hif-1α in multiple cell types is the induction of metabolic reprogramming, to shift metabolism to glycolysis in conditions of limited oxygen availability [110]. Despite the known role of Hif-1α in promoting glycolysis in multiple cell types, it is noteworthy that Hif-1α is not required for the initiation of aerobic glycolysis in activated T cells; instead, c-Myc is uniquely required for this metabolic shift [111]. Despite this fact, it is clear that Hif-1α can play an important role in promoting optimal metabolism in the case of Th17 differentiation [97]. However, more detailed and precise studies will be

required to elicit the specific requirements for Hif-1α in T cell metabolism.

Hif-1α in non-conventional T cell subsets

To date, the role of Hif-1α in the regulation of other T cell subsets including γδ T cells, IELs, and NKT cells remains unknown. Given the presence of these cell types at sites of physiologic hypoxia (e.g., γδ T cells residing in the skin and IELs lining the anaerobic environment of the gut lumen), it is extremely likely that hypoxic signaling will also regulate their function and that this is a greatly understudied, but potentially fascinating avenue of investigation.

Hypoxic signaling in T cells beyond Hif-1α

Despite recent advances in our understanding of the role of Hif-1α in regulating T cells, our current understanding of hypoxic regulation in T cells remains in its relative infancy. In particular, there is a complete lack of information on hypoxic regulation in T cells mediated by Hif-2α, NF-kB, or hypoxic-elicited miRNAs. Furthermore, the common use of Hif-1β in both hypoxic signaling and the aryl hydrocarbon receptor pathway, which regulates Th17 differentiation and IL-10 production [112, 113], raises the possibility of cross talk in regulating T cell function. In a broader sense, it will also be important to understand conserved and unique features of the hypoxic T cell response, how it integrates with T cell receptor signaling pathways, and whether different T cell subsets have varied hypoxic responsiveness [114].

Therapeutic potential of the hypoxic pathway

Because of the known contributions of hypoxia to a wide variety of pathologies, there is strong interest in developing therapies that modulate hypoxic signaling. By disrupting the HIF proteolytic pathway, researchers have identified compounds that activate HIF and hypoxic signaling [115]. HIF activators have been used in a variety of in vivo models, where they have been shown to potentiate innate antibacterial defense mechanisms [116] and to limit inflammation in models of inflammatory bowel disease and LPS-induced shock [117–120]. HIF activators appear capable of triggering multiple tissue protective pathways that protect epithelial cells from apoptosis, elicit an M2-like macrophage phenotype, and induce IL-10 production in B cells [119, 120]. Although there has been concern about the use of HIF activators systemically due to the potential for deleterious side effects in humans, recent phase I studies have shown that systemic treatment with HIF activators is safe and potentially useful in boosting EPO levels in patients with end-stage renal disease [121].

Despite these early positive therapeutic results, HIF activators are not beneficial in all situations. For example, while HIF activation protects against LPS-induced shock, it is deleterious in mice subjected to polymicrobial sepsis following cecal ligation puncture [120]. Another caveat of current HIF activators is that many are prolyl hydroxylase (PHD) inhibitors. Since PHDs are capable of modulating the activity of multiple pathways including NF-kB [122], these compounds likely have pleiotropic effects beyond activation of HIF, which can lead to potentially unpredictable side effects.

The search for possible HIF inhibitors has led to the identification of a number of molecules that impair HIF at various transcriptional and translational stages (ranging from protein stabilization to dimerization to DNA binding) [123]. One significant hurdle for the use of these compounds, however, is the lack of specificity of these compounds for specific blockade of HIF (such as digoxin, acriflavine, and 17-AAG) identified to date [124–126]. Despite these caveats, therapies designed to interfere with HIF signaling in tumors are already in clinical trials in humans to assess antitumor efficacy [127].

With the emergence of various HIF modulators, it is possible that these compounds may have efficacy in regulating T cell responses either through the activation or inhibition of hypoxic signaling. While a more complete understanding of the role of hypoxic signaling in T cells will be required to make full use of these compounds, early experiments in mice using two distinct HIF activators have demonstrated that these compounds can locally increase the abundance of regulatory T cells and reduce inflammation in the context of inflammatory bowel disease [128]. As further insights into the hypoxic regulation of T cells come to light, pharmacologic manipulation of hypoxic signaling may afford new opportunities to modify T cell function in order to decrease inflammation, improve vaccine efficacy, and potentially have efficacy in resolving clinically important chronic viral and bacterial infections.

Conclusions

Oxygen is an important microenvironmental factor that is dynamically regulated in conditions of health and disease. The migratory pattern of naïve and effector T cells exposes T cells to a variety of oxygen tensions. Notably, from development to maturity, T cells traffic through tissues in which oxygen tensions are low enough to induce hypoxic signaling and Hif-1α expression. In addition, data demonstrating that Hif-1α is activated following T cell receptor stimulation and under certain cytokine-driven

differentiation pathways (e.g., Th17 differentiation) suggest that Hif-1α might have an important role in T cell activation and differentiation programs beyond its role in coordinating responses to hypoxia. One major challenge in understanding the consequence of hypoxic signaling in T cells will be to further define the dynamic interplay between oxygen availability, T cell differentiation programs, and T cell function. With the increasing availability of sophisticated genetic models to interrogate the genetic contribution of hypoxic signaling specifically in T cells, combined with emerging pharmacologic tools that manipulate the hypoxic response, the field is poised to gain major new insights into how hypoxic signaling in T cells is regulated both in the context of immunity and a variety of disease processes.

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