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Immunologic Consequences of Hypoxia during Critical Illness

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Abstract

Hypoxia and immunity are highly intertwined at the clinical, cellular, and molecular level. The prevention of tissue hypoxia and modulation of systemic inflammation are cornerstones of daily practice in the Intensive Care Unit. Potentially, immunologic effects of hypoxia may contribute to outcome and represent possible therapeutic targets. Hypoxia and activation of downstream signaling pathways result in enhanced innate immune responses, aimed to augment pathogen clearance. On the other hand, hypoxia also exerts anti-inflammatory and tissue-protective effects in lymphocytes and other tissues. Although human data on the net immunologic effects of hypoxia and pharmacological modulation of downstream pathways are limited, preclinical data support the concept of tailoring the immune response through modulation of the oxygen status or pharmacological modulation of hypoxia-signaling pathways in critically ill patients.

Introduction

Optimization of oxygenation to prevent tissue hypoxia is one of the cornerstones of critical care. Concurrently, in the majority of patients admitted to the Intensive Care Unit (ICU), inflammatory processes take place which may affect outcome. As hypoxia and immunity are highly interdependent at the molecular, cellular and clinical level, immunologic effects of hypoxia may represent therapeutic targets in critically ill patients. At the cellular level, hypoxia activates distinct hypoxia-signaling pathways, including a group of transcription factors known as Hypoxia Inducible Factors and adenosine signaling. *In vitro* and animal studies have shown that these pathways are involved in modulation of inflammatory responses, and animal studies have demonstrated that these pathways are relevant to inflammatory conditions that are frequently encountered in critically ill patients, such as sepsis^{1,2} and lung injury^{3,4}. In addition, inflammatory conditions are frequently

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characterized by tissue hypoxia due to enhanced metabolic demand as well as decreased metabolic substrates resulting from edema, microthrombi, and atelectasis, in turn causing “inflammatory hypoxia”^{5,6}. As such, aiming for specific tissue oxygenation levels could be favorable in a range of inflammatory conditions in critically ill patients. Alternatively, these effects may also be achieved with pharmacologic interventions targeting hypoxia-signaling pathways.

In the present review, we provide an overview of the immunologic consequences of hypoxia. We focus on *in vitro*, animal, and human studies concerning inflammatory conditions relevant to the critically ill patient, including a discussion of oxygen-dependent signaling pathways and intermediate signaling systems (e.g. the HIF system and adenosine metabolism)^{2,7}. Furthermore we discuss the clinical potential of intervening in these mechanisms, including evidence on potential drawbacks of hyperoxia, feasibility of therapeutic *permissive hypoxia*, and pharmacologic therapies that act on oxygen-dependent pathways. The role of hypoxia and HIFs outside the scope of inflammatory conditions in critically ill patients are reviewed elsewhere^{2,7-9}.

Immunologic effects of hypoxia

Evidence for immunologic effects of hypoxia have mainly been established in *in vitro* studies using myeloid cells (Table 1)¹⁰⁻¹². Long-term hypoxia has been shown to represent an inflammatory stimulus in itself, as prolonged hypoxia results in production of cytokines in a human macrophage cell line¹³. In addition, hypoxia increases the production of pro-inflammatory cytokines upon stimulation with the toxins lipopolysaccharide (LPS)- or Phytohaemagglutinin (PHA) in primary human mononuclear cells^{14,15}. In contrast, other studies have demonstrated that hypoxia skews the pro-inflammatory character (M1-like) of macrophages towards an anti-inflammatory M2-like phenotype^{16,17}. In addition to these contradictory findings, these *in vitro* studies are difficult to interpret, as the control condition is usually room air, which has a higher PaO₂ compared to physiologic tissue PaO₂. Nevertheless, these *in vitro* studies demonstrate that oxygenation exerts immunologic effects, although the direction of this response may depend on the cell type and activation state.

Healthy volunteers subjected to hypoxia *in vivo* display enhanced *ex vivo* neutrophil chemotaxis, phagocytosis, and reactive oxygen species production¹⁸, and increased activity of the key inflammatory transcription factor nuclear factor KB (NFKB) in monocytes¹⁹. Furthermore, exposure of healthy subjects to high altitude hypoxia (SaO₂ 75-90%) for four days results in elevated plasma levels of the pro-inflammatory interleukin (IL)-6^{20,21}, while shorter periods of hypoxia do not induce such systemic responses^{22,23} (Table 1). Taken together, *in vivo*, prolonged hypoxia increases inflammatory responses of myeloid cells *ex vivo* and elicits a systemic immune response.

Concerning the underlying mechanisms, hypoxia *in vitro* induces an expansive cascade of cellular processes, regulated by oxygen-sensitive pathways consisting of prolyl hydroxylases (PHDs), the transcription factors Hypoxia Inducible Factors (HIFs) and NFKB, adenosine signaling pathways, and other oxygen-sensitive processes. These cellular mechanisms

provide adaptation towards conditions of limited oxygen availability, and each pathway contributes in different ways to the immunologic effects of hypoxia. This may explain why hypoxia causes both, pro- and anti-inflammatory, as well as tissue-protective effects, as further detailed below.

Regulation of Hypoxia Inducible Factor-1 α

HIFs represent a group of transcription factors that mediate a plethora of cellular adaptations in response to hypoxia²⁴. HIFs are heterodimers consisting of HIF- β and one of three O₂-dependent transcriptionally active α subunits: HIF-1 α , HIF-2 α , and HIF-3 α , of which HIF-1 α is the most widely studied isoform. The cellular mechanisms responsible for the regulation of HIF-1 α protein stabilization and signaling under normoxic, hypoxic, and inflammatory conditions are detailed in Figure 1. Under normoxic conditions, the oxygen-dependent prolyl hydroxylases (PHD)-1, PHD2, and PHD3, and the asparaginyl-hydroxylase factor-inhibiting HIF (FIH) hydroxylate HIF-1 α , after which hydroxylated HIF-1 α binds to the Von Hippel Lindau (VHL) complex. Binding of HIFs to VHL ultimately results in ubiquitination and degradation in the proteasome. Under hypoxic conditions, the oxygen-dependent hydroxylases are inactive, which prevents degradation of HIF-1 α . As such, hypoxia regulates HIF-1 α in a posttranslational manner. A second, oxygen-independent, posttranslational mechanism of HIF-1 α regulation involves Heat Shock Protein (HSP) 90. HSPs are key players in the response to cellular stress, functioning as chaperone proteins that facilitate conformation, localization, and function of a diversity of proteins. HSP90 blocks the oxygen-independent degradation of HIF-1 α and thereby results in stabilization of HIF-1 α ^{25–27}. Furthermore, HSP90 binding to HIF-1 α facilitates coupling with HIF β and subsequent transactivation²⁷.

Finally, the transcription and translation of HIF-1 α is increased by inflammatory stimuli. Therefore, hypoxia, cellular stress, and inflammation (synergistically) enhance HIF-1 α stabilization^{2,7}.

HIF-1 α stabilization facilitates transcription of >100 hypoxia-responsive genes²⁸, many of which result in hypoxia-adaptation, e.g. erythropoietin and vascular endothelial growth factor²⁹. Although the autoregulatory system of HIF-1 α has not been fully elucidated, there appears to be a negative feedback system³⁰. *In vitro*, hypoxia induces HIF-1 α expression in a dose-dependent fashion, but prolonged hypoxia results in downregulation of HIF-1 α , mediated by a microRNA which targets HIF (aHIF), of which levels increase over time under hypoxic conditions³¹. In contrast with *in vitro* data, where hypoxia has only been shown to prevent HIF-1 α degradation, hypoxia *in vivo* stimulates transcription of HIF-1 α , followed by a decrease to baseline levels, possibly resulting from the aHIF-mediated negative feedback³². Human studies revealed a large interindividual variability in leukocyte HIF-1 α expression³³ and downstream target gene expression³⁴ in response to hypoxia, implicating phenotypical differences in HIF regulation.

Involvement of HIF-1 α and adenosine signaling in the immunologic effects of hypoxia

The molecular interplay between hypoxia, HIFs and NF κ B

The regulation of HIF-1 α and NF κ B, the latter considered the master regulator of inflammatory responses, is highly intertwined^{35,36}. In the inactivated state, NF κ B is bound to the inhibitory protein I κ B α in the cytosol. Inflammatory stimuli, but also other signals activate the enzyme I κ B kinase (IKK), resulting in phosphorylation of I κ B α . Subsequently, NF κ B translocates into the nucleus and an inflammatory response characterized by production of inflammatory cytokines is generated³⁷. Another downstream effect of NF κ B activity is enhanced HIF-1 α transcription^{38–40}. Conversely, HIF-1 α activity enhances NF κ B activity by increasing abundance of IKK and the NF κ B subunit p65^{39,41}. Moreover, hypoxia prevents PHD-dependent IKK-degradation⁴². *In vitro* studies confirmed this effect, as combined inhibition of PHD-1 and FIH enhanced basal NF κ B activity in a HIF-1 α independent fashion⁴³. Paradoxically, PHD-1 and FIH inhibition suppress NF κ B activity under inflammatory conditions⁴³. These data illustrate that there is extensive interplay between hypoxia, oxygen-dependent hydroxylases, HIF-1 α , and NF κ B. Furthermore, as alluded to before, effects are dependent on the cellular activation state.

Cellular and in vivo immunologic effects of HIF-1 α

At the cellular level, HIF-1 α stabilization in immune cells results in a differentiated response, highly depending on the cell type. In neutrophils, the induction of β_2 -integrin involved in epithelial neutrophil binding⁴⁴, regulation of pathogen-binding neutrophil extracellular traps, and anti-bacterial activity⁴⁵ are all HIF-1 α -dependent⁴⁵. HIF-1 α stabilization inhibits apoptosis of macrophages and neutrophils^{41,46}, is involved in the differentiation of monocytes to macrophages as well as in macrophage maturation⁴⁷. HIF-1 α also results in increased expression of Toll-like receptor (TLR)-4⁴⁸ as well as enhanced macrophage phagocytosis⁴⁹ and bacterial killing⁵⁰.

A wide diversity of animal studies using cell-specific transgenic knockout mice and pharmacologic HIF-1 α modulation also demonstrate the cell type-specific effects of HIF-1 α . Myeloid HIF-1 α knockout mice have a higher morbidity in streptococcal skin infections than their wild type littermates, which indicates that HIF-1 α in myeloid cells is essential to mount an inflammatory response required to clear local infection⁵⁰. In severe systemic inflammation induced by LPS (to mimic Gram negative infection)⁵¹ or lipoteichoic acid and peptidoglycan (to mimic Gram positive infection)⁵², myeloid HIF-1 α deficient mice display an attenuated inflammatory response, associated with less tissue damage and improved survival⁵¹. In accordance, HIF-1 α gain of function results in an overwhelming inflammatory response in sterile and bacterial peritonitis, with aggravated organ damage and impaired survival¹. As such, in myeloid cells, HIF-1 α is essential for the generation of an effective inflammatory response to clear infections, while simultaneously, HIF-1 α overexpression leads to the clinical picture of the early, pro-inflammatory phase of sepsis in mice.

In contrast to the pro-inflammatory effects observed in myeloid cells, HIF-1 α activity induces anti-inflammatory and tissue-protective effects in lymphocytes. For instance, HIF-1 α induction results in increased numbers of regulatory T-cells, with subsequent tissue protection due to attenuation of inflammation⁵³. Furthermore, in a murine bacterial peritonitis model, T-cell-specific HIF-1 α deficiency results in increased levels of pro-inflammatory cytokines⁵⁴. Suggestive of anti-inflammatory effects of HIF-1 α in B-cells, PHD-inhibition with dimethyloxallyl glycine (DMOG) prior to LPS-administration in mice resulted in enhanced IL-10 production by B1-cells, which skewed macrophages towards an anti-inflammatory M2-like phenotype⁵⁵. Moreover, other studies demonstrate that the transcriptional program that drives anti-inflammatory regulatory T-cell differentiation is under the control of HIF via the induction of the HIF-target gene FoxP3⁵³.

Apart from effects in dedicated immune cells, HIF-1 α stabilization also exerts immunologic effects in other cells, e.g. intestinal and alveolar epithelium and myocytes. Pharmacologic stabilization of HIF-1 α through PHD-inhibition in murine chemical-induced colitis results in reduced levels of TNF α , IL-6, and IL-1 β , while levels of anti-inflammatory IL-10 increase⁵⁶, and clinical outcome improves^{57,58}. Similarly, pharmacologic PHD-inhibition in ventilator induced lung injury results in HIF-1 α dependent reduced lung injury and prolonged survival, whereas HIF-1 α inhibition aggravates lung injury and shortened survival⁴. The tissue-protective effects of HIF-1 α are also involved in protection against ischemic injury. For instance, myocardial protection by remote ischemic preconditioning is dependent on increased IL-10 production mediated through HIF-1 α ^{59,60}, and myocardial HIF-1 α expression mediates a metabolic switch to glycolysis, which is crucial for adaptation to ischemia⁶¹. An overview of the immunologic effects of PHD-inhibition in *in vitro* and animal studies is provided in Table 2^{62–74}.

Altogether, HIF-1 α activity in myeloid cells is involved in the orchestration of immune responses aimed at pathogen clearance, whereas HIF-1 α activity in lymphocytes, epithelium, and myocytes induces anti-inflammatory and tissue protective effects (an overview is provided in Figure 2). Although these opposing effects may seem contradictory, studies in the field of oncology have shown that myeloid HIF-1 α activity suppresses T-cell responses⁷⁵. Therefore, it is conceivable that, in the context of inflammation and infection, local interplay between different immune cells is required to optimize infection control and simultaneously prevent tissue damage⁷⁶.

HIF-1 α in sepsis

The role of HIF-1 α in sepsis is of particular interest, as inflammation and tissue hypoxia often co-exist, the latter due to a mismatch of oxygen demand and availability. The immunologic host response during early sepsis is characterized by (over)production of pro-inflammatory cytokines, which is aimed at pathogen clearance but also results in the clinical syndrome of septic shock. However, an anti-inflammatory reaction is mounted simultaneously, presumably to curtail the pro-inflammatory response and thereby prevent collateral tissue damage. When too pronounced and/or sustained, this anti-inflammatory response results in a profoundly suppressed state of the immune system. It is increasingly recognized that this phenomenon, known as “sepsis-induced immunoparalysis”, renders

patients more vulnerable towards secondary infections and is a major contributor to late mortality in septic patients⁷⁷.

Based on the data described earlier, HIF-1 α activity may enhance pro-inflammatory effects and innate immune functions, which could be beneficial in sepsis-induced immunoparalysis. This concept is supported by the observation that endotoxin tolerance, which bears similarities to sepsis-induced immunoparalysis, was partially reversed by chronic mild hypoxia in mice⁷⁸. However, this single animal study does not fully reflect the complex dynamics of HIF-1 α during human sepsis. Furthermore, it needs to be emphasized that the abovementioned studies on (the interplay between) inflammation and hypoxia have been conducted *in vitro* and in animals. The translation from animal studies to the human situation is an important topic of debate^{79,80}. Fortunately, two recent observational studies in sepsis patients have increased our understanding of the dynamics of HIF-1 α during sepsis. In one of these, samples were obtained within 2-4 hours after admission, and HIF-1 α mRNA expression in monocytes was increased⁸¹. Furthermore, HIF-1 α induced the negative Toll-like receptor regulator IRAK-M, resulting in immunosuppression⁸¹. In contrast, the other study found reduced leukocytic HIF-1 α protein and mRNA expression, but samples were obtained at later time points (i.e. within 24 hours after admission)⁸². Although one has to be cautious when interpreting data from preclinical work in the context of clinical patient studies, it could be envisioned that the early pro-inflammatory response drives increased HIF-1 α expression, resulting in the induction of negative regulators such as IRAK-M to counteract excessive inflammation, ultimately resulting in reduced HIF-1 α levels later in the course of sepsis.

Tissue-protective and anti-inflammatory effects through the adenosine pathway

Hypoxia can also exert anti-inflammatory and tissue-protective effects through the adenosine pathway, of which some elements have been reported to be HIF-1 α dependent^{83,84}.

Cellular distress (e.g. hypoxia⁸⁵) results in increased availability of the adenosine progenitors adenosine triphosphate (ATP) and adenosine diphosphate (ADP)⁸⁶. Hypoxia leads to upregulation of CD39 (ecto-apyrase)^{87,88}, which converts ATP and ADP into AMP, and to HIF-1 α -dependent upregulation of CD73 (5'ecto-nucleotidase), which converts AMP into adenosine⁸³. The tissue-protective effects of these enzymes have been demonstrated in studies using knockout mice. For example, mice lacking either CD39 or CD73 display increased morbidity and mortality after inflammatory or ischemic injury⁸⁹⁻⁹¹. Correspondingly, genetic overexpression of HIF-1 α results in increased epithelial expression of CD73 and improves outcome in murine chemically induced colitis⁹². Finally, hypoxia increases the expression of the adenosine 2A (A2A) and A2B receptors, the latter in a HIF-1 α dependent manner⁸⁴. Stimulation of these receptors results in systemic anti-inflammatory effects in murine models of ischemia-reperfusion⁹⁰, hypoxia⁹³, and inflammation⁹⁴. Furthermore, permissive hypoxia (FiO₂ 10%) attenuated lung damage and improved survival in a murine model of acute lung injury in an A2A receptor-dependent manner⁹⁵, and induction of the A2B-receptor in type 1 alveolar cells during ventilator-induced lung injury was shown to be dependent on HIF-1 α ³. Similarly, hypoxic

preconditioning protected mice from liver ischemia and reperfusion injury in an A2B receptor-dependent manner⁹⁶.

The limited human data available substantiate that hypoxia results in enhanced adenosine availability. For instance, exposure to short-term hypoxia (20 min, SaO₂ 80%) in healthy volunteers increases plasma adenosine levels⁹⁷. Furthermore, several experimental human studies have demonstrated anti-inflammatory effects of adenosine signaling, as intravenous adenosine administration⁹⁸ as well as oral treatment with the adenosine uptake inhibitor dipyridamole⁹⁹ attenuated the pro-inflammatory IL-6 response during experimental human endotoxemia, and dipyridamole treatment also augmented anti-inflammatory IL-10 production⁹⁹. However, increased adenosine availability in these latter studies was not induced by hypoxia. Finally, a proof-of-concept clinical study revealed that interferon- β -1a enhances CD73 expression in human lung tissue and that administration of this cytokine to ARDS patients is associated with reduced IL-6 and IL-8 levels as well as improved PaO₂/Fio₂ ratios and survival¹⁰⁰.

In addition to HIF-1 α , NF κ B, and adenosine metabolism and signaling pathways, other oxygen-sensitive transcription factors have been identified although the exact oxygen-dependent mechanisms and downstream effects are not fully elucidated (reviewed in¹⁰¹).

A schematic overview of the complex interplay between hypoxia and inflammation is depicted in Figure 2.

Hypoxia in critically ill patients

Hypoxic respiratory failure is a common condition in ICU patients, with an incidence of 22-33%^{102,103}, depending on the definition (usually the need for mechanical ventilation and/or a PaO₂/FiO₂ ratio of <300 mmHg¹⁰²⁻¹⁰⁴), and is associated with a mortality of 31-52%¹⁰²⁻¹⁰⁴. A subcategory of hypoxic respiratory failure is Acute Respiratory Distress Syndrome (ARDS), comprising 3-70% of patients with respiratory failure¹⁰²⁻¹⁰⁴. ARDS severity can be classified according to the Berlin definitions as either mild (200-300 mmHg), moderate (100-200 mmHg), or severe (<100 mmHg), with mortality ranging from 32 to 65%¹⁰⁴⁻¹¹⁰. It is important to differentiate between the diagnosis of hypoxic respiratory failure (i.e. an indication for intubation and mechanical ventilation due to hypoxia) and actual hypoxia (i.e. low PaO₂), as patients with hypoxic respiratory failure can have normal PaO₂ levels. The occurrence of hypoxia (i.e. PaO₂ <80 mmHg) at ICU admission is frequent (40%)¹¹¹, and in a retrospective cohort study in Dutch ICU patients, hypoxia at ICU admission or during ICU stay was shown to be associated with increased mortality, even after correction for disease severity and other confounders¹¹¹. The association between hypoxia at ICU admission and increased mortality was confirmed in a similar analysis in Australian and New Zealand ICU patients¹¹². However, these studies are observational in nature, and although efforts have been made to eliminate bias, confounding factors may still play a role. Therefore, these studies cannot be used to guide oxygen therapy. Currently, oxygenation targets for critically ill patients are lacking. Since the landmark study on tidal volumes in ARDS, a target of 55-80 mmHg or SaO₂ 88-95% is used in ARDS studies¹¹³, even though there is no solid evidence supporting these targets¹¹⁴.

Results of clinical trials on the effects of oxygenation in ICU patients are necessary to determine optimal oxygenation targets in diverse subsets of patients. Currently, the O₂-ICU study randomizes ICU patients with systemic inflammation to either a target PaO₂ of 120 mmHg or 75 mmHg ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02321072) Identifier NCT02321072). The Hyper2S study ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01722422) Identifier NCT01722422), in which patients with septic shock were randomized in a 2×2 fashion to normoxia (SaO₂ 88–95%) vs. FiO₂ 100% for 24 hours and resuscitation with isotonic saline vs. hypertonic saline, was preliminary terminated because of a borderline significant increase in mortality in the hyperoxic/hypertonic group¹¹⁵. Additionally, the AVOID trial has showed that normoxic patients with ST-elevation myocardial infarction treated with supplemental oxygen display increased creatine kinase levels and myocardial infarct sizes compared with normoxic patients who did not receive additional oxygen¹¹⁶. The putative harmful effects of hyperoxia have instigated further exploration of the safety and feasibility of conservative oxygenation targets. Two before-after studies in mechanically ventilated ICU patients applied SaO₂ targets of 90–92%¹¹⁷ and 92–95%¹¹⁸, respectively, which was not associated with in adverse outcomes. The safety and feasibility of a conservative oxygenation strategy was recently affirmed by a randomized controlled pilot study comparing a liberal oxygenation strategy (SpO₂ > 96%) with a conservative strategy (SpO₂ 88–92%)¹¹⁹. These results may pave the way for the exploration of a personalized oxygen target to influence inflammation in critically ill patients.

The translation of preclinical data on hypoxia and inflammation towards treatment in critically ill patients

As illustrated by animal studies and the limited clinical data available, hypoxia and downstream signaling pathways may represent important and amendable factors in the pathophysiology of inflammatory conditions in critically ill patients such as sepsis and lung injury. However, many hurdles still have to be taken before we can translate these insights into clinical practice. The host-responses in inflammatory conditions in critically ill patients are complex, with considerable interindividual differences and changes over time. Nevertheless, it is conceivable that modulating the immune response towards a targeted, personalized, favorable immunologic phenotype, e.g. immunostimulatory therapy in sepsis-induced immunoparalysis or anti-inflammatory therapy in acute lung injury, may be of clinical benefit¹²⁰. As many specific therapeutic target interventions have failed to show benefit in clinical trials¹²¹, it would be naïve to assume that targeting hypoxia-dependent pathways is “the magic bullet”. Nonetheless, optimization of all amendable parameters to tailor the inflammatory host-response towards a more preferable profile should still be considered. As oxygen management is daily practice in the ICU, the immunologic effects of oxygenation should therefore also be taken into account as a means of optimizing host-responses.

Although grossly based on *in vitro* and animal data, oxygenation-dependent immunomodulatory strategies could be envisioned as either pursuing a nullification of hypoxia-induced immunologic effects by preventing hypoxia, or enhancing immunologic effects of hypoxia by preventing hyperoxia or even permit or induce hypoxia. For example, animal data suggests that averting hyperoxia and even permitting hypoxia is beneficial in

acute lung injury⁹⁵, apart from prevention of direct oxygen toxicity. Naturally, intentional or permissive hypoxia as a therapeutic strategy is only expedient when safety margins are taken into account, especially as PaO₂ targets would be at the steep part of the oxygen-hemoglobin dissociation curve. As previously proposed, a suitable oxygenation monitoring and control system should use real-time data on pulse oximetry, tissue oxygenation, and arterial oxygen tension to achieve a predefined oxygenation¹²², and should naturally be extensively tested for safety, feasibility, and efficacy.

However, caution is warranted, as there is an association between long-term neurocognitive impairment and the amount of time that ARDS patients were hypoxic (i.e. SaO₂ <90%)¹²³. Therefore, short-term benefits of hypoxia, i.e. putative therapeutic effects in inflammation, and long-term effects, i.e. neurocognitive impairment need to be carefully weighed.

Alongside hypoxia, or if permissive hypoxia does not prove to be feasible, HIF-1 α -mediated effects could also be pursued through pharmacologic inhibition of PHDs. The PHD-inhibitor FG-4497 increased HIF-1 α stabilization in mice, with subsequent resistance of stem cells to irradiation¹²⁴, improved kidney transplantation survival¹²⁵ and attenuated TNF α expression and weight loss during colitis⁵⁷. A comparable PHD inhibitor (FG-2216) resulted in increased plasma EPO levels in hemodialysis patients¹²⁶, however, due to a case of fatal hepatic necrosis and other patients developing abnormal liver enzyme tests, the FDA suspended this clinical trial and further development was discontinued¹²⁷. Nonetheless, clinical trials with new drugs targeting PHDs for treatment of anemia in patients with chronic renal disease and dialysis are currently being performed²⁴. Whether pharmacologic HIF stimulation affects the immune response in humans has not been established yet. Additionally, the frequently used PHD-inhibitor Dimethyloxallyl Glycine (DMOG) and the aforementioned FG compounds are pan-hydroxylase inhibitors, and are not specific for HIF-1 α stabilization, which may lead to undesired effects. For example, DMOG also stabilizes HIF-2 α , which results in increased EPO levels^{57,58} and could thus cause polycythemia. This can be circumvented by more specific PHD-inhibitors, such as the selective PHD-1 inhibitor AKB-4924, and/or local instead of systemic drug delivery⁶⁹.

Taken together, although the concept of tailoring the immune response through oxygenation or pharmacological modulation of hypoxia-signaling pathways is tempting, the question remains if this approach is feasible and will result in clinical benefits for the patient. Therefore, studies assessing the putative therapeutic potential of these effects are highly warranted. Furthermore, immunomodulatory therapy in inflammatory conditions in the ICU still faces many challenges. For example, anti-inflammatory strategies in e.g. sepsis have been unsuccessful in the past¹²¹, possibly because they render patients increased vulnerability to secondary infections, although it might also be due to the profound heterogeneity of this patient population. Immunostimulatory therapy to prevent and/or reverse immunoparalysis is currently under investigation for sepsis⁷⁷. Meanwhile, a search for markers identifying the current “immune status” of ICU patients is ongoing, and may result in better identification of patients that could benefit from immunomodulating therapy¹²⁸.

Conclusion

There is extensive interplay between hypoxia and the immune system. Hypoxia and inflammation synergistically induce HIF-1 α stabilization, resulting in cellular effects directed towards augmented pathogen clearance, of interest, simultaneously anti-inflammatory and tissue-protective mechanisms occur, for instance through enhanced adenosine metabolism and signaling. The net effect of these effects is highly dependent on the cell type and activation state. Insights in these hypoxia-driven mechanisms promote the concept of personalizing oxygenation targets to tailor the immune response in inflamed critically ill patients. However, the development of such strategies requires exploration of the putative effects of hypoxia on the immune response in humans *in vivo*, as these data are currently lacking. Furthermore, additional studies into pharmacologic HIF-1 α stabilizers and agents acting on the adenosine pathway are required. In any case, the optimal PaO₂ and oxygen delivery in critically ill patients is likely to depend on diagnosis and comorbidities, and clinicians should be aware that their oxygen therapy may not only affect saturation, but also the inflammatory host response. As clinical guidelines on optimal oxygenation are currently not present, ongoing clinical trials exploring the feasibility of liberal vs. restrictive oxygenation are highly warranted and currently in progress; these could further pave the way towards individualized oxygenation therapy.

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Figure 1. HIF-1α regulation and signaling under normoxic, hypoxic, and inflammatory conditions

HIF-1α subunits are constantly produced but rapidly degraded under normoxic conditions. Several pathways of HIF-1α regulation have been described. First, under normoxic conditions, HIF-1α subunits are rapidly hydroxylated by oxygen-dependent prolyl-hydroxylase domain enzymes (PHDs), which are subsequently captured by the ubiquitin ligase Von Hippel-Lindau (VHL) protein and degraded by the proteasome. Second, the oxygen-dependent asparaginyl hydroxylase Factor Inhibiting HIF (FIH) hydroxylates a conserved asparaginyl residue, preventing the recruitment of co-activators p300 and CBP, in turn inhibiting dimerization with HIFβ. During O₂-deficiency, PHD and FIH activity decrease, resulting in accumulation of HIF-1α subunits in the cytosol. The Receptor for Activated C Kinase 1 (RACK1) and Heat Shock Protein 90 (HSP90) regulate HIF-1α in an oxygen-independent manner: RACK facilitates oxygen-independent proteasomal degradation of HIF-1α, while HSP90 competes with RACK, thereby stabilizing HIF-1α, and facilitates its transactivation.

Upon accumulation, HIF-1α is co-activated by p300/CBP and dimerizes with HIFβ to form stable HIF1αβ dimers. These dimers translocate to the nucleus and bind to Hypoxia Response Elements (HREs) in promoter enhancer regions of genes, resulting in

transcriptional activity. HIF-1 α stabilization results in transcription of many (>100) hypoxia responsive genes. As FIH remains active at lower oxygen concentrations than PHDs, FIH suppresses the activity of HIF-1 α proteins that escape destruction during moderate hypoxia. Not only hypoxia, but also exposure to bacteria and bacterial products such as LPS results in HIF-1 α accumulation.

Figure 2. The interaction between hypoxia and inflammation

Hypoxia enhances the immune response, and is an inflammatory stimulus by itself. Hypoxia leads to cellular stabilisation of Hypoxia Inducible Factor 1α (HIF-1α), resulting in a synergistic effect with the key inflammatory transcription factor NFκB. In addition, inflammation enhances transcription and translation of HIF-1α, leading to a synergistic effect in case of hypoxia and inflammation. In myeloid cells, such as neutrophils and monocytes, HIF-1α activity exerts pro-inflammatory effects, aimed at clearance of pathogens. Conversely, in many other cells, such as T-cells, pulmonary and interstitial epithelium, and myocytes, HIF-1α activity has anti-inflammatory effects. Furthermore, hypoxia exerts anti-inflammatory effects through the adenosine pathway, as it increases the availability of adenosine progenitors adenosine triphosphate (ATP) and adenosine diphosphate (ADP), upregulates the converting enzymes CD39 and CD73 to enhance adenosine production, and increases the expression of the anti-inflammatory adenosine 2A and 2B receptors (A2A and A2B). The upregulation of CD73 and adenosine receptors are HIF-1α-dependent.

In vitro and (pre)clinical studies on the effects of hypoxia on immunity**Table 1**

	Reference	Model	Inflammatory stimulus/model	Oxygen intervention	Timing oxygen intervention	Inflammatory effect of oxygen intervention
<i>in vitro</i>	14	Primary human monocytes	LPS or PMA	1% oxygen	24h	↑ TNFα and IL-1β
	13	Monocyte/macrophage cell line (THP-1)	–	1% or 9% oxygen	Up to 24h	↑ TNFα
	15	Primary human monocytes	PHA	2% oxygen	16h and 40h	↑ IL-2, IL-4, IL-6, and IFNγ ↓ IL-10
	44	Monocyte/macrophage cell line (U937)	–	2.8% oxygen	24h	↑ adhesion of leukocytes to endothelium
	10	Primary human dendritic cells, monocyte (MM6), endothelial (HMEC-1) and intestinal epithelial (Caco-2) cell lines	–	2% oxygen	6h	↑ TLR2 and TLR6
animal model (pre) clinical human studies	22	Murine bone marrow-derived dendritic cells	LPS	1% oxygen	24h	↑ costimulatory molecules, TNFα, and IL6
	17	Rat alveolar macrophages	LPS	1.3% oxygen	1.5h	↓ TNFα and IL-1β
	16	Murine peritoneal macrophages and monocyte cell lines (U937 and THP-1)	LPS	<0.3% oxygen	24h	↑ TNFα
	16	TG-elicited murine peritoneal macrophages and LPS-primed monocyte cell lines (U937 and THP-1)	LPS	<0.3% oxygen	24h	↓ TNFα
	78	Mice	LPS i.p. at days 11 and 27	12% oxygen	28 days	↑ TNFα
	20	Healthy volunteers	none	Altitude hypoxia (4350m, SaO2 78.6-83.4%)	4 days	↑ IL-6
	21	Healthy volunteers	none	Altitude hypoxia (3458-4559m, SaO2 75-90%)	4 days	↑ IL-6, IL-1RA, and CRP
	23	Healthy volunteers	none	Altitude hypoxia (4500m)	2 h per day for 7 consecutive days	Neutrophilia, ↑ neutrophilic superoxide production
	18	Healthy volunteers	<i>ex vivo</i> stimulation of neutrophils with fMLP	12% oxygen	2 h	↑ chemotaxis, phagocytosis and ROS production

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Reference	Model	Inflammatory stimulus/model	Oxygen intervention	Timing oxygen intervention	Inflammatory effect of oxygen intervention
19	Healthy volunteers	<i>ex vivo</i> stimulation of T-cells and monocytes with PMA, phagocytosis of zymosan	SAO ₂ 78%	2 h	= cytokines, ↑ neutrophil phagocytosis
11	Healthy volunteers	none	SAO ₂ 80%	1h per day for 10 consecutive days	= cytokines
12	Healthy volunteers	none	11% oxygen	30 or 60 min	= cytokines

LPS: Lipopolysaccharide; PMA: phorbol myristate acetate; h: hours; TNFα: Tumor Necrosis Factor alpha; IL-1β: Interleukin-1β; PHA: Phytohemagglutinin; IL-2: interleukin-2; IL-4: Interleukin-4; IL-6: Interleukin-6; IFNγ: Interferon γ; IL-10: Interleukin-10; TLR2: Toll Like Receptor 2; TLR6: Toll Like Receptor 6; i.p.: intraperitoneal; IL-1RA: Interleukin-1 receptor antagonist; CRP: C-Reactive Protein; fMLP: N-formyl-Met-Leu-Phe; ROS: reactive oxygen species; SAO₂: arterial oxygen saturation; min: minutes. Control condition was room air unless specified otherwise, pressure is normobaric unless specified otherwise. References are listed in the online supplement.

Table 2

In vitro and preclinical studies on the effects of PHD-inhibitors on immunity

	Reference	Model	Inflammatory stimulus/model	Intervention	Inflammatory effect of PHD-inhibition
<i>in vitro</i>	62	Microglial cell line (BV2)	LPS	EDHB	↓ mRNA TNF α and IL-6
	63	Keratinocyte cell line (HaCaT)	LPS	AKB-4924	↑ VEGF, IL-6, IL-8
	64	Monocyte/macrophage cell line (U937) and neutrophils from healthy donors	Various Gram positive and negative bacteria	AKB-4924	↑ bactericidal activity
animal model	65	Endothelial cell line (EA32)	TNF α	DMOG	↓ VCAM-1
	66	Macrophage cell line (RAW264.7)	LPS	DMOG	↓ TNF α
	67	HeLa cell line	IL-1 β	DMOG	↓ NF κ B activity
	57	Mice	TNBS (chemical colitis)	FG-4497	↓ colonic TNF α , weight loss, histological inflammation
	58	Mice	DSS (chemical colitis)	DMOG	↓ colonic IL-1 β , TNF α , IL-12, IL-6, disease activity index, weight loss, histological inflammation
	74	Rats	DNBS (chemical colitis)	DMOG	↓ neutrophil infiltration
	68	Mice	TNBS and DSS (chemical colitis)	TRC160334	↓ disease activity index, weight loss, histological inflammation
	56	Mice	TNBS (chemical colitis)	AKB-4924	↓ serum IL-1 β , TNF α , IL-6, weight loss, disease activity ↑ IL-10
	69	Mice	TNBS (chemical colitis)	AKB-4924	↓ colonic IL-1 β , TNF α , IL-12, IL-6, weight loss, histological inflammation
	70	Mice	TNF α ARE ^{+/+} mice (spontaneous chronic terminal ileitis)	DMOG	↓ histological inflammation
	71	Mice	LPS intraperitoneal (endotoxemic shock)	DMOG	↓ TNF α , mortality ↑ IL-10
	4	Mice	Ventilator induced lung injury	DMOG	↓ BAL MPO, pulmonary edema ↑ gas exchange, survival time
	64	Mice	<i>S. Aureus</i> (cutaneous infection)	AKB-4924	↓ lesion size, bacterial load, disease severity
	72	Mice	<i>E. Coli</i> (urinary tract infection)	AKB-4924	↓ bacterial load, IL-1 β , IL-6, KC, MPO activity
	62	Mice	MPTP (neurotoxicity)	EDHB	↓ striatal IL-6
	73	Rabbits	LPS and methylprednisolone (osteonecrosis)	EDHB	↓ osteonecrosis

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LPS: Lipopolysaccharide; EDHB: ethyl-3,4-dihydroxybenzoate; TNF α : Tumor Necrosis Factor α ; IL-6: Interleukin-6; VEGF: Vascular Endothelial Growth Factor; IL-8: Interleukin-8; DMOG: dimethylxaloylglycine; VCAM-1: Vascular Cell Adhesion Molecule-1; IL-1 β : Interleukin-1 β ; NF κ B: Nuclear Factor of kappa-light-chain-enhancer of activated B cells; TNBS: 2,4,6-trinitrobenzene sulfonic acid; DSS: dextran sulfate sodium; IL-12: Interleukin-12; DNBS: dinitrobenzene sulfonic acid; IL-10: Interleukin-10; TNF ARE/+ mice: mice with gene targeted alterations in untranslated region of TNF α mRNA leading to development of severe ileitis; MPO: Myeloperoxidase; BAL: bronchial alveolar lavage; *S Aureus*: *Staphylococcus Aureus*; *E Coli*: *Escherichia Coli*; KC: keratinocyte-derived chemokine; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine