Immunologic Consequences of Hypoxia during Critical Illness

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ABSTRACT

Hypoxia and immunity are highly intertwined at clinical, cellular, and molecular levels. 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 antiinflammatory and tissue-protective effects in lymphocytes and other tissues. Although human data on the net immunologic effects of hypoxia and pharmacologic modulation of downstream pathways are limited, preclinical data support the concept of tailoring the immune response through modulation of the oxygen status or pharmacologic modulation of hypoxia-signaling pathways in critically ill patients. (Anesthesiology 2016; 125:237-49)

In the current 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 critically ill patients, including a discussion of oxygen-dependent signaling pathways and intermediate signaling systems (e.g., the hypoxia-inducible factor [HIF] system and adenosine metabolism). 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 is reviewed elsewhere.

Immunologic Effects of Hypoxia

Evidence for immunologic effects of hypoxia has mainly been established in in vitro studies using myeloid cells (table 1). 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 line. In addition, hypoxia increases the production of proinflammatory cytokines upon stimulation with the toxins lipopolysaccharide or phytohemagglutinin in primary human mononuclear cells. In contrast, other studies
Table 1. *In Vitro* and (Pre)Clinical Studies on the Effects of Hypoxia on Immunity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Model</th>
<th>Inflammatory Stimulus/Model</th>
<th>Oxygen Intervention</th>
<th>Timing Oxygen Intervention</th>
<th>Inflammatory Effect of Oxygen Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em></td>
<td>13</td>
<td>Primary human monocytes</td>
<td>Lipopolysacharide or PMA</td>
<td>1% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Monocyte/macrophage cell line (THP-1)</td>
<td>—</td>
<td>1 or 9% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Up to 24 h</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Primary human monocytes</td>
<td>Phytohemagglutinin</td>
<td>2% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>16 h and 40 h</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Monocyte/macrophage cell line (U937)</td>
<td>—</td>
<td>2.8% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Murine bone marrow–derived dendritic cells</td>
<td>Lipopolysacharide</td>
<td>1% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Rat alveolar macrophages</td>
<td>Lipopolysacharide</td>
<td>1.3% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.5 h</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>Murine peritoneal macrophages and monocytic cell lines (U937 and THP-1)</td>
<td>Lipopolysacharide</td>
<td>&lt; 0.3% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>TG-elicited murine peritoneal macrophages and lipopolysacharide–primed monocytic cell lines (U937 and THP-1)</td>
<td>Lipopolysacharide</td>
<td>&lt; 0.3% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>24 h</td>
</tr>
<tr>
<td><em>Animal model</em></td>
<td>20</td>
<td>Mice</td>
<td>Lipopolysacharide intraperitoneal at days 11 and 27</td>
<td>12% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>28 d</td>
</tr>
<tr>
<td><em>(Pre)clinical human studies</em></td>
<td>21</td>
<td>Healthy volunteers</td>
<td>None</td>
<td>Altitude hypoxia (4,350 m; SaO₂, 78.6 to 83.4%)</td>
<td>4 d</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>Healthy volunteers</td>
<td>None</td>
<td>Altitude hypoxia (3458–4559 m; SaO₂, 75 to 90%)</td>
<td>4 d</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Healthy volunteers</td>
<td>None</td>
<td>Altitude hypoxia (4,500 m)</td>
<td>2 h per day for 7 consecutive days</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Healthy volunteers</td>
<td>Ex vivo stimulation of neutrophils with fMLP</td>
<td>12% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>Healthy volunteers</td>
<td>Ex vivo stimulation of T cells and monocytes with PMA, phagocytosis of zymosan</td>
<td>SaO₂, 78%</td>
<td>2 h</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Healthy volunteers</td>
<td>None</td>
<td>SaO₂, 80%</td>
<td>1 h/day for 10 consecutive days</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Healthy volunteers</td>
<td>None</td>
<td>11% O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>30 or 60 min</td>
</tr>
</tbody>
</table>

Control condition was room air unless specified otherwise and pressure is normobaric unless specified otherwise.

CRP = C-reactive protein; fMLP = n-formyl-Met-Leu-Phe; HMEC-1 = human microvascular endothelial cell; IFNγ = interferon γ; MM6 = mono mac 6 human monocytic cell; PMA = phorbol myristate acetate; ROS = reactive oxygen species; SaO₂ = arterial oxygen saturation; TG = thioglycollate; TLR2 = toll-like receptor 2; TLR6 = toll-like receptor 6; TNFα = tumor necrosis factor alpha.
have demonstrated that hypoxia skews the proinflammatory character (M1-like) of macrophages toward an anti-inflammatory M2-like phenotype. 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 production and increased activity of the key inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in monocytes. Furthermore, exposure of healthy subjects to high-altitude hypoxia (arterial oxygen saturation [SaO2], 75 to 90%) for 4 days results in increased plasma levels of the proinflammatory interleukin-6, while shorter periods of hypoxia do not induce such systemic responses (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 HIFs and NF-κB, adenosine signaling pathways, and other oxygen-sensitive processes. These cellular mechanisms provide adaptation toward 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 antiinflammatory, as well as tissue-protective effects, as further detailed below.

### Regulation of HIF-1α

HIFs represent a group of transcription factors that mediate a plethora of cellular adaptations in response to hypoxia. HIFs are heterodimers consisting of HIF-β and one of the three oxygen-dependent transcriptionally active α subunits: HIF-1α, HIF-2α, and HIF-3α, of which HIF-1α is the most widely studied isoform. The cellular mechanisms responsible for the regulation of HIF-1α protein stabilization and signaling under normoxic, hypoxic, and inflammatory conditions are detailed in figure 1. Under normoxic conditions, the oxygen-dependent PHD-1, PHD2, and PHD3 and the asparaginyl-hydroxylase factor–inhibiting HIF (FIH) hydroxylate HIF-1α, after which hydroxylated HIF-1α binds to the Von Hippel–Lindau complex. Binding of HIFs to Von Hippel–Lindau ultimately results in ubiquitination and degradation in the proteasome. Under hypoxic conditions, the oxygen-dependent hydroxylases are inactive, which prevents degradation of HIF-1α. As such, hypoxia regulates HIF-1α in a posttranslational manner. A second, oxygen-independent, posttranslational mechanism of HIF-1α 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-1α and thereby results in stabilization of HIF-1α. Furthermore, HSP90 binding to HIF-1α facilitates coupling with HIFβ and subsequent transactivation.

Finally, the transcription and translation of HIF-1α are increased by inflammatory stimuli. Therefore, hypoxia, cellular stress, and inflammation (synergistically) enhance HIF-1α stabilization. HIF-1α stabilization facilitates transcription of more than 100 hypoxia-responsive genes, many of which result in hypoxia adaptation, e.g., erythropoietin and vascular endothelial growth factor. Although the autoregulatory system of HIF-1α has not been fully elucidated, there appears to be a negative feedback system. In vitro, hypoxia induces HIF-1α expression in a dose-dependent fashion, but prolonged hypoxia results in down-regulation of HIF-1α, mediated by a micro-RNA, which targets HIF (αHIF), of which levels increase over time under hypoxic conditions. In contrast to in vitro data, where hypoxia has only been shown to prevent HIF-1α degradation, hypoxia in vivo stimulates transcription of HIF-1α, followed by a decrease to baseline levels, possibly resulting from the aHIF-mediated negative feedback. Human studies revealed a large interindividual variability in leukocyte HIF-1α expression and downstream target gene expression in response to hypoxia, implicating phenotypical differences in HIF regulation.

### Involvement of HIF-1α and Adenosine Signaling in the Immunologic Effects of Hypoxia

**The Molecular Interplay among Hypoxia, HIFs, and NF-κB**

The regulation of HIF-1α and NF-κB, the latter considered the master regulator of inflammatory responses, is highly intertwined. In the inactivated state, NF-κB is bound to the inhibitory protein IκBα in the cytosol. Not only inflammatory stimuli but also other signals, activate the enzyme IκB kinase (IKK), resulting in phosphorylation of IκBα. Subsequently, NF-κB translocates into the nucleus, and an inflammatory response characterized by production of inflammatory cytokines is generated. Another downstream effect of NF-κB activity is enhanced HIF-1α transcription. Conversely, HIF-1α activity enhances NF-κB activity by increasing abundance of IKK and the NF-κB subunit p65. Moreover, hypoxia prevents PHD-dependent IKK degradation. In vitro studies confirmed this effect, as combined inhibition of PHD-1 and FIH enhanced basal NF-κB activity in a HIF-1α-independent fashion. Paradoxically, PHD-1 and FIH inhibition suppress NF-κB activity under inflammatory conditions. These data illustrate that there is extensive interplay between hypoxia, oxygen-dependent hydroxylases, HIF-1α, and NF-κB. Furthermore,
Immunologic Consequences of Hypoxia

Cellular and In Vivo Immunologic Effects of HIF-1α

At the cellular level, HIF-1α stabilization in immune cells results in a differentiated response, highly depending on the cell type. In neutrophils, the induction of β₂-integrin involved in epithelial neutrophil binding, regulation of pathogen-binding neutrophil extracellular traps, and antibacterial activity are all HIF-1α dependent. HIF-1α stabilization inhibits apoptosis of macrophages and neutrophils and is involved in the differentiation of monocytes to macrophages as well as in macrophage maturation. HIF-1α also results in increased expression of toll-like receptor 4 as well as in enhanced macrophage phagocytosis and bacterial killing.

A wide diversity of animal studies using cell-specific transgenic knockout mice and pharmacologic HIF-1α modulation also demonstrate the cell type–specific effects of HIF-1α. Myeloid HIF-1α knockout mice have a higher morbidity in

as alluded to before, effects are dependent on the cellular activation state.

**Fig. 1.** Hypoxia-inducible factor (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 coactivators P300 and cAMP-response element–binding protein (CBP), in turn inhibiting dimerization with HIFβ. During oxygen deficiency, PHD and FIH activities 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 coactivated by P300/CBP and dimerizes with HIFβ to form stable HIF-1αβ 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-1α stabilization results in transcription of many (greater than 100) hypoxia responsive genes. As FIH remains active at lower oxygen concentrations than PHDs, FIH suppresses the activity of HIF-1α proteins that escape destruction during moderate hypoxia. Not only hypoxia but also exposure to bacteria and bacterial products such as lipopolysaccharide (LPS) results in HIF-1α accumulation. NF-κB = nuclear factor of kappa-light-chain-enhancer of activated B cells; TLR = toll-like receptor.
staphylococcal skin infections than their wild-type littermates, which indicates that HIF-1α in myeloid cells is essential to mount an inflammatory response required to clear local infection.51 In severe systemic inflammation induced by lipopolysaccharide (to mimic Gram-negative infection)52 or lipoteichoic acid and peptidoglycan (to mimic Gram-positive infection),53 myeloid HIF-1α-deficient mice display an attenuated inflammatory response, associated with less tissue damage and improved survival.52 In accordance, HIF-1α gain of function results in an overwhelming inflammatory response in sterile and bacterial peritonitis, with aggravated organ damage and impaired survival. As such, in myeloid cells, HIF-1α is essential for the generation of an effective inflammatory response to clear infections, while simultaneously, HIF-1α overexpression leads to the clinical picture of the early, proinflammatory phase of sepsis in mice.

In contrast to the proinflammatory effects observed in myeloid cells, HIF-1α activity induces antiinflammatory and tissue-protective effects in lymphocytes. For instance, HIF-1α induction results in increased numbers of regulatory T cells, with subsequent tissue protection due to attenuation of inflammation. Further, the in murine bacterial peritonitis model, T-cell-specific HIF-1α deficiency results in increased levels of proinflammatory cytokines.55 Suggestive of antiinflammatory effects of HIF-1α in B cells, PHD inhibition with dimethyl-oxalylglycine before lipopolysaccharide administration in mice resulted in enhanced interleukin-10 production by B1 cells, which skewed macrophages toward an antiinflammatory M2-like phenotype. Moreover, other studies demonstrate that the transcriptional program that drives antiinflammatory regulatory T-cell differentiation is under the control of the HIF-target gene FoxP3.

Apart from effects in dedicated immune cells, HIF-1α stabilization also exerts immunologic effects in other cells, e.g., intestinal and alveolar epithelium and myocytes. Pharmacologic stabilization of HIF-1α through PHD inhibition in murine chemical-induced colitis results in reduced levels of TNFα, interleukin-6, and interleukin-1β, while levels of antiinflammatory interleukin-10 increase and clinical outcome improves. Similarly, pharmacologic PHD inhibition in ventilator-induced lung injury results in HIF-1α-dependent reduced lung injury and prolonged survival, whereas HIF-1α inhibition aggravates lung injury and shortened survival. The tissue-protective effects of HIF-1α are also involved in protection against ischemic injury. For instance, myocardial protection by remote ischemic preconditioning is dependent on increased interleukin-10 production mediated through HIF-1α, and myocardial HIF-1α expression mediates a metabolic switch to glycolysis, which is crucial for adaptation to ischemia. An overview of the immunologic effects of PHD inhibition in vivo and animal studies is provided in table 2.

Altogether, HIF-1α activity in myeloid cells is involved in the orchestration of immune responses aimed at pathogen clearance, whereas HIF-1α activity in lymphocytes, epithelium, and myocytes induces antiinflammatory and tissue protective effects (an overview is provided in fig. 2). Although these opposing effects may seem contradictory, studies in the field of oncology have shown that myeloid HIF-1α activity suppresses T-cell responses. 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 damage.

**HIF-1α in Sepsis**

The role of HIF-1α in sepsis is of particular interest, as inflammation and tissue hypoxia often coexist, the latter due to a mismatch of oxygen demand and availability. The immunologic host response during early sepsis is characterized by (over)production of proinflammatory cytokines, which is aimed at pathogen clearance, but also results in the clinical syndrome of septic shock. However, an antiinflammatory reaction is mounted simultaneously, presumably to curtail the proinflammatory response and thereby prevent collateral tissue damage. When too pronounced and/or sustained, this antiinflammatory 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 to secondary infections and is a major contributor to late mortality in septic patients.

Based on the data described earlier, HIF-1α activity may enhance proinflammatory 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 mice. However, this single animal study does not fully reflect the complex dynamics of HIF-1α 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. Fortunately, two recent observational studies in sepsis patients have increased our understanding of the dynamics of HIF-1α during sepsis. In one of these, samples were obtained within 2 to 4 h after admission, and HIF-1α mRNA expression in monocytes was increased. Furthermore, HIF-1α induced the negative toll-like receptor regulator interleukin-1 receptor-associated kinase M, resulting in immunosuppression. In contrast, the other study found reduced leukocytic HIF-1α protein and mRNA expression, but samples were obtained at later time points (i.e., within 24 h after admission). 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 proinflammatory response drives increased HIF-1α expression, resulting in the induction of negative regulators such as interleukin-1 receptor-associated kinase M to
counteract excessive inflammation, ultimately resulting in reduced HIF-1α levels later in the course of sepsis.

Tissue-protective and Antiinflammatory Effects through the Adenosine Pathway

Hypoxia can also exert antiinflammatory and tissue-protective effects through the adenosine pathway, of which some elements have been reported to be HIF-1α dependent. Hypoxia can also exert antiinflammatory and tissue-protective effects through the adenosine pathway, of which some elements have been reported to be HIF-1α dependent.

Cellular distress (e.g., hypoxia) results in increased availability of the adenosine progenitors adenosine triphosphate and adenosine diphosphate. Hypoxia leads to up-regulation of CD39 (ectoapyrase), which converts adenosine triphosphate and adenosine diphosphate into adenosine monophosphate, and to HIF-1α–dependent up-regulation of CD73 (5′-ectonucleotidase), which converts adenosine monophosphate into adenosine.

Table 2. In Vitro and Preclinical Studies on the Effects of PHD Inhibitors on Immunity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Model</th>
<th>Inflammatory Stimulus/Model</th>
<th>Intervention</th>
<th>Inflammatory Effect of PHD Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>Microglial cell line (BV2)</td>
<td>Lipopolysaccharide</td>
<td>EDHB</td>
<td>↓ mRNA, TNFα, and interleukin-6</td>
</tr>
<tr>
<td>64</td>
<td>Keratinocyte cell line (HaCaT)</td>
<td>Lipopolysaccharide</td>
<td>AKB-4924</td>
<td>↑ VEGF, interleukin-6, interleukin-8</td>
</tr>
<tr>
<td>65</td>
<td>Monocyte/macrophage cell line (U937) and neutrophils from healthy donors</td>
<td>Various Gram-positive and negative bacteria</td>
<td>AKB-4924</td>
<td>↑ Bacterial activity</td>
</tr>
<tr>
<td>66</td>
<td>Endothelial cell line (SA32)</td>
<td>TNFα</td>
<td>Dimethylxalylglycine</td>
<td>↓ VCAM-1</td>
</tr>
<tr>
<td>67</td>
<td>Macrophage cell line (RAW264.7)</td>
<td>Lipopolysaccharide</td>
<td>Dimethylxalylglycine</td>
<td>↓ TNFα</td>
</tr>
<tr>
<td>45</td>
<td>HeLa cell line</td>
<td>Interleukin-1β</td>
<td>Dimethylxalylglycine</td>
<td>↑ VEGF, interleukin-6, interleukin-8</td>
</tr>
<tr>
<td>58</td>
<td>Mice</td>
<td>TNBS (chemical colitis)</td>
<td>FG-4497</td>
<td>↓ Colonic TNFα, weight loss, histologic inflammation</td>
</tr>
<tr>
<td>59</td>
<td>Mice</td>
<td>DSS (chemical colitis)</td>
<td>Dimethylxalylglycine</td>
<td>↓ Colonic interleukin-1β, TNFα, interleukin-12, interleukin-6, disease activity index, weight loss, histologic inflammation</td>
</tr>
<tr>
<td>74</td>
<td>Rats</td>
<td>DNBS (chemical colitis)</td>
<td>Dimethylxalylglycine</td>
<td>↑ Neutrophil infiltration</td>
</tr>
<tr>
<td>68</td>
<td>Mice</td>
<td>TNBS and DSS (chemical colitis)</td>
<td>TRC160334</td>
<td>↓ Disease activity index, weight loss, histologic inflammation</td>
</tr>
<tr>
<td>57</td>
<td>Mice</td>
<td>TNBS (chemical colitis)</td>
<td>AKB-4924</td>
<td>↓ Serum interleukin-1β, TNFα, interleukin-6, weight loss, disease activity</td>
</tr>
<tr>
<td>69</td>
<td>Mice</td>
<td>TNBS (chemical colitis)</td>
<td>AKB-4924</td>
<td>↓ Interleukin-10</td>
</tr>
<tr>
<td>70</td>
<td>Mice</td>
<td>TNF-ARE/α mice (spontaneous chronic terminal ileitis)</td>
<td>Dimethylxalylglycine</td>
<td>↓ Histologic inflammation</td>
</tr>
<tr>
<td>71</td>
<td>Mice</td>
<td>Lipopolysaccharide intraarterial (endotoxemic shock)</td>
<td>Dimethylxalylglycine</td>
<td>↓ TNFα, mortality</td>
</tr>
<tr>
<td>72</td>
<td>Mice</td>
<td>Staphylococcus aureus (cutaneous infection)</td>
<td>Dimethylxalylglycine</td>
<td>↑ Interleukin-10</td>
</tr>
<tr>
<td>45</td>
<td>Mice</td>
<td>Ventilator-induced lung injury</td>
<td>Dimethylxalylglycine</td>
<td>↑ BAL, MPO, pulmonary edema</td>
</tr>
<tr>
<td>65</td>
<td>Mice</td>
<td>Escherichia coli (urinary tract infection)</td>
<td>AKB-4924</td>
<td>↑ Gas exchange, survival time</td>
</tr>
<tr>
<td>72</td>
<td>Mice</td>
<td>Lipopolysaccharide and methylprednisolone (osteonecrosis)</td>
<td>AKB-4924</td>
<td>↑ Bacterial load, interleukin-1β, interleukin-6, KC, myeloperoxidase activity</td>
</tr>
<tr>
<td>63</td>
<td>Mice</td>
<td>MPTP (neurotoxicity)</td>
<td>EDHB</td>
<td>↑ Striatal interleukin-6</td>
</tr>
<tr>
<td>73</td>
<td>Rabbits</td>
<td>Lipopolysaccharide and methylprednisolone (osteonecrosis)</td>
<td>EDHB</td>
<td>↑ Osteonecrosis</td>
</tr>
</tbody>
</table>

BAL = bronchial alveolar lavage; DNBS = dinitrobenzene sulfonic acid; DSS = dextran sulfate sodium; EDHB = ethyl-3,4-dihydroxybenzoate; KC = keratinocyte-derived chemokine; MPO = myeloperoxidase; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NF-κB = nuclear factor of kappa-light-chain-enhancer of activated B cells; PHDI = prolyl hydroxylase; TNBS = 2,4,6-trinitrobenzene sulfonic acid; TNFα = tumor necrosis factor alpha; TNFΔARE/α mice = mice with gene targeted alterations in untranslated region of TNFα mRNA leading to development of severe ileitis; VCAM-1 = vascular cell adhesion molecule-1; VEGF = vascular endothelial growth factor.
The limited human data available substantiate that hypoxia results in enhanced adenosine availability. For instance, exposure to short-term hypoxia (20 min; $\text{SaO}_2$, 80%) in healthy volunteers increases plasma adenosine levels.96 Furthermore, several experimental human studies have demonstrated antiinflammatory effects of adenosine signaling, as intravenous adenosine administration97 as well as oral treatment with the adenosine uptake inhibitor
Hypoxic respiratory failure is a common condition in ICU patients, with an incidence of 22 to 33%, depending on the definition (usually the need for mechanical ventilation and/or a PaO2/FIO2 ratio of less than 300 mmHg), and is associated with a mortality of 31 to 52%. ARDS severity can be classified according to the Berlin definitions as mild (200 to 300 mmHg), moderate (100 to 200 mmHg), or severe (less than 100 mmHg), with mortality ranging from 32 to 65%. 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 less than 80 mmHg) at ICU admission is frequent (40%), 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 confounders. The association between hypoxia at ICU admission and increased mortality was confirmed in a similar analysis in Australian and New Zealand ICU patients. 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 to 80 mmHg or Sao2 88 to 95% is used in ARDS studies, even though there is no solid evidence supporting these targets.

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 or 75 mmHg (Clinicaltrials.gov Identifier NCT02321072). The Hyper2S study (Clinicaltrials.gov Identifier NCT01722422), in which patients with septic shock were randomized in a 2 × 2 fashion to normoxia (Sao2, 88 to 95%) versus FIO2 100% for 24 h and resuscitation with isotonic saline versus hypertonic saline, was preliminary terminated because of a borderline significant increase in mortality in the hyperoxic/hypertonic group. Additionally, the Air Versus Oxygen in ST-Segment Elevation Myocardial Infarction trial has shown that normoxic patients with ST-elevation myocardial infarction treated with supplemental oxygen exhibit increased creatine kinase levels and myocardial infarct sizes compared with normoxic patients who did not receive additional oxygen. 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 to 92% and 92 to 95%, respectively, which was not associated with 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, greater than 96%) with a conservative strategy (SpO2, 88 to 92%). 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 toward 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 toward a targeted, personalized, favorable immunologic phenotype, e.g., immunostimulatory therapy in sepsis-induced immunoparalysis or antiinflammatory therapy in acute lung injury, may be of clinical benefit. As many specific therapeutic target interventions have failed to show benefit in clinical trials, 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 toward a more preferable profile should still be considered. As oxygen management is a 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 hypoxia or even permitting or inducing hypoxia. For example, animal data suggest that averting hyperoxia and even permitting hypoxia is beneficial in acute lung injury, 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 oxygenation 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, less than 90%). 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-1α-mediated effects could also be pursued through pharmacologic inhibition of PHDs. The PHD inhibitor FG-4497 increased HIF-1α stabilization in mice, with subsequent resistance of stem cells to irradiation, improved kidney transplantation survival, and attenuated TNFα expression and weight loss during colitis. A comparable PHD inhibitor (FG-2216) resulted in increased plasma erythropoietin levels in hemodialysis patients; however, due to a case of fatal hepatic necrosis and other patients developing abnormal liver enzyme tests, the U.S. Food and Drug Administration suspended this clinical trial, and further development was discontinued. Nonetheless, clinical trials with new drugs targeting PHDs for treatment of anemia in patients with chronic renal disease and dialysis are currently being performed. Whether pharmacologic HIF stimulation affects the immune response in humans has not been established yet. Additionally, the frequently used PHD inhibitor dimethylxaloylglycine and the aforementioned FG compounds are pan-hydroxylase inhibitors and are not specific for HIF-1α stabilization, which may lead to undesired effects. For example, dimethylxaloylglycine also stabilizes HIF-2α, which results in increased erythropoietin levels 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 delivery.

Taken together, although the concept of tailoring the immune response through oxygenation or pharmacologic 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, antimicrobial strategies in sepsis have been unsuccessful in the past, 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 sepsis. Meanwhile, a search for markers identifying the current “immune status” of ICU patients is ongoing and may result in better identification of patients who could benefit from immunomodulating therapy.

Conclusion

There is extensive interplay between hypoxia and the immune system. Hypoxia and inflammation synergistically induce HIF-1α stabilization, resulting in cellular effects directed toward augmented pathogen clearance, of interest, simultaneously antiinflammatory 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 into 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 on pharmacologic HIF-1α stabilizers and agents acting on the adenosine pathway are required. In any case, the optimal PaO₂ and oxygen delivery in critically ill patients are likely to depend on diagnosis and comorbidities, and clinicians should be aware that their oxygen therapy may affect not only 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 versus restrictive oxygenation are highly warranted and currently in progress; these could further pave the way toward individualized oxygenation therapy.

Research Support

Supported by grants R01-DK097075, R01-HL092188, R01-HL098294, PO1-HL114457, and R01-HL119837 from the National Institutes of Health, Bethesda, Maryland (to Dr. Eltzschig); a Ph.D. grant from the Radboud Centre for Infectious Diseases, Nijmegen, The Netherlands, and a Young Investigator Grant from the Dutch Society of Anesthesiology, Utrecht, The Netherlands (to Dr. Kox).

Competing Interests

The authors declare no competing interests.
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