

# Immunologic Consequences of Hypoxia during Critical Illness

Harmke D. Kiers, M.D., Gert-Jan Scheffer, M.D., Ph.D., Johannes G. van der Hoeven, M.D., Ph.D., Holger K. Eltzschig, M.D., Ph.D., Peter Pickkers, M.D., Ph.D., Matthijs Kox, Ph.D.

## 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**)

**O**PTIMIZATION of oxygenation to prevent tissue hypoxia is one of the cornerstones of critical care. Currently, 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 molecular, cellular, and clinical levels, 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<sup>1,2</sup> and lung injury.<sup>3,4</sup> In addition, inflammatory conditions are frequently 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.”<sup>5,6</sup> 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 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).<sup>2,7</sup> 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.<sup>2,7-9</sup>

## Immunologic Effects of Hypoxia

Evidence for immunologic effects of hypoxia has mainly been established in *in vitro* studies using myeloid cells (table 1).<sup>10-12</sup> 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.<sup>14</sup> In addition, hypoxia increases the production of proinflammatory cytokines upon stimulation with the toxins lipopolysaccharide or phytohemagglutinin in primary human mononuclear cells.<sup>13,15</sup> In contrast, other studies

This article is featured in “This Month in Anesthesiology,” page 1A. Figures 1 and 2 were enhanced by Annemarie B. Johnson, C.M.I., Medical Illustrator, Vivo Visuals, Winston-Salem, North Carolina. James C. Eisenach, M.D., served as Editor-in-Chief for this article.

Submitted for publication September 17, 2015. Accepted for publication February 29, 2016. From the Departments of Intensive Care Medicine (H.D.K., J.G.v.d.H., P.P., M.K.) and Anesthesiology (H.D.K., G.-J.S., M.K.), and the Radboud Centre for Infectious Diseases (H.D.K., J.G.v.d.H., P.P., M.K.), Radboud University Medical Center, Nijmegen, The Netherlands; and Organ Protection Program, Department of Anesthesiology, University of Colorado School of Medicine, Aurora, Colorado (H.K.E.).

Copyright © 2016, the American Society of Anesthesiologists, Inc. Wolters Kluwer Health, Inc. All Rights Reserved. Anesthesiology 2016; 125:237-49

**Table 1.** *In Vitro* and (Pre)Clinical Studies on the Effects of Hypoxia on Immunity

Reference	Model	Inflammatory Stimulus/Model	Oxygen Intervention	Timing Oxygen Intervention	Inflammatory Effect of Oxygen Intervention
13	Primary human monocytes	Lipopolysaccharide or PMA	1% O <sub>2</sub>	24 h	↑ TNF $\alpha$ and interleukin-1 $\beta$
14	Monocyte/macrophage cell line (THP-1)	—	1 or 9% O <sub>2</sub>	Up to 24 h	↑ TNF $\alpha$
15	Primary human monocytes	Phytohemagglutinin	2% O <sub>2</sub>	16 h and 40 h	↑ Interleukin-2, interleukin-4, interleukin-6, and IFN $\gamma$ ↓ Interleukin-10
16	Monocyte/macrophage cell line (U937)	—	2.8% O <sub>2</sub>	24 h	↑ Adhesion of leukocytes to endothelium
10	Primary human dendritic cells, monocytic (MM6), endothelial (HMEC-1), and intestinal epithelial (Caco-2) cell lines	—	2% O <sub>2</sub>	6 h	↑ TLR2 and TLR6
17	Murine bone marrow-derived dendritic cells	Lipopolysaccharide	1% O <sub>2</sub>	24 h	↑ Costimulatory molecules, TNF $\alpha$ , and interleukin-6
18	Rat alveolar macrophages	Lipopolysaccharide	1.3% O <sub>2</sub>	1.5 h	↓ TNF $\alpha$ and interleukin-1 $\beta$
19	Murine peritoneal macrophages and monocytic cell lines (U937 and THP-1)	Lipopolysaccharide	< 0.3% O <sub>2</sub>	24 h	↑ TNF $\alpha$
19	TG-elicited murine peritoneal macrophages and lipopolysaccharide-primed monocytic cell lines (U937 and THP-1)	Lipopolysaccharide	< 0.3% O <sub>2</sub>	24 h	↓ TNF $\alpha$
20	Mice	Lipopolysaccharide intraperitoneal at days 11 and 27	12% O <sub>2</sub>	28 d	↑ TNF $\alpha$
21	Healthy volunteers	None	Altitude hypoxia (4,350 m; SaO <sub>2</sub> , 78.6 to 83.4%)	4 d	↑ Interleukin-6
22	Healthy volunteers	None	Altitude hypoxia (3458–4559 m; SaO <sub>2</sub> , 75 to 90%)	4 d	↑ Interleukin-6, interleukin-1RA, and CRP
23	Healthy volunteers	None	Altitude hypoxia (4,500 m)	2 h per day for 7 consecutive days	↑ Neutrophilia ↑ Neutrophilic superoxide production
24	Healthy volunteers	<i>Ex vivo</i> stimulation of neutrophils with fMLP	12% O <sub>2</sub>	2 h	↑ Chemotaxis, phagocytosis, and ROS production
25	Healthy volunteers	<i>Ex vivo</i> stimulation of T cells and monocytes with PMA, phagocytosis of zymosan	SaO <sub>2</sub> 78%	2 h	= Cytokines ↑ Neutrophil phagocytosis
11	Healthy volunteers	None	SaO <sub>2</sub> 80%	1 h/day for 10 consecutive days	= Cytokines
12	Healthy volunteers	None	11% O <sub>2</sub>	30 or 60 min	= Cytokines

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; IFN $\gamma$  = interferon  $\gamma$ ; MM6 = mono mac 6 human monocytic cell; PMA = phorbol myristate acetate; ROS = reactive oxygen species; SaO<sub>2</sub> = arterial oxygen saturation; TG = thioglycollate; TLR2 = toll-like receptor 2; TLR6 = toll-like receptor 6; TNF $\alpha$  = tumor necrosis factor alpha.

have demonstrated that hypoxia skews the proinflammatory character (M1-like) of macrophages toward an anti-inflammatory M2-like phenotype.<sup>18,19</sup> 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  $P_{aO_2}$  compared to physiologic tissue  $P_{aO_2}$ . 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<sup>24</sup> and increased activity of the key inflammatory transcription factor nuclear factor of kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) in monocytes.<sup>25</sup> Furthermore, exposure of healthy subjects to high-altitude hypoxia (arterial oxygen saturation [ $SA_{O_2}$ ], 75 to 90%) for 4 days results in increased plasma levels of the proinflammatory interleukin-6,<sup>21,22</sup> while shorter periods of hypoxia do not induce such systemic responses<sup>17,23</sup> (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- $\kappa$ B, 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-1 $\alpha$

HIFs represent a group of transcription factors that mediate a plethora of cellular adaptations in response to hypoxia.<sup>26</sup> HIFs are heterodimers consisting of HIF- $\beta$  and one of the three oxygen-dependent transcriptionally active  $\alpha$  subunits: HIF-1 $\alpha$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$ , of which HIF-1 $\alpha$  is the most widely studied isoform. The cellular mechanisms responsible for the regulation of HIF-1 $\alpha$  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-1 $\alpha$ , after which hydroxylated HIF-1 $\alpha$  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-1 $\alpha$ . As such, hypoxia regulates HIF-1 $\alpha$  in a posttranslational manner. A second, oxygen-independent, posttranslational mechanism of HIF-1 $\alpha$  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 $\alpha$  and thereby results in stabilization of HIF-1 $\alpha$ .<sup>27-29</sup> Furthermore, HSP90 binding to HIF-1 $\alpha$  facilitates coupling with HIF $\beta$  and subsequent transactivation.<sup>29</sup>

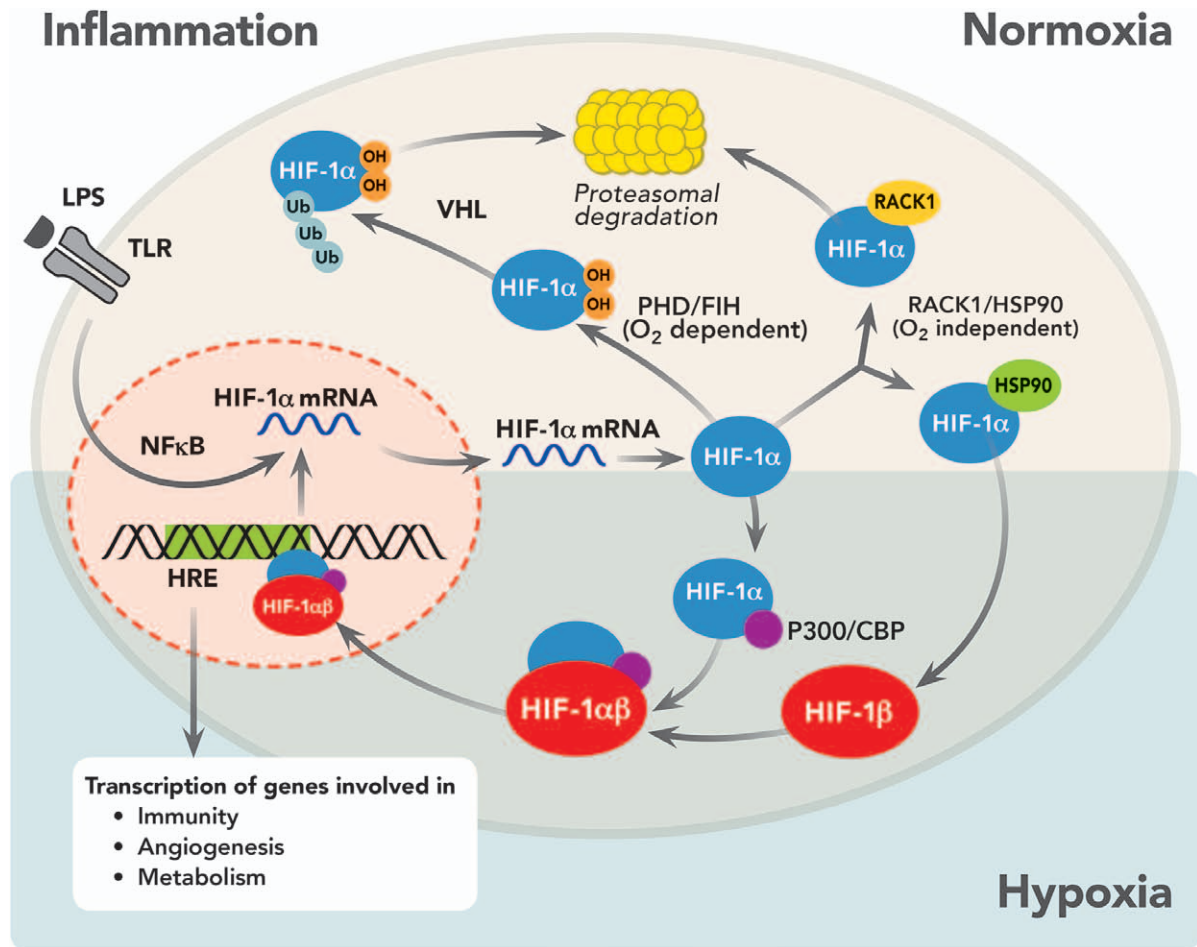
Finally, the transcription and translation of HIF-1 $\alpha$  are increased by inflammatory stimuli. Therefore, hypoxia, cellular stress, and inflammation (synergistically) enhance HIF-1 $\alpha$  stabilization.<sup>2,7</sup>

HIF-1 $\alpha$  stabilization facilitates transcription of more than 100 hypoxia-responsive genes,<sup>30</sup> many of which result in hypoxia adaptation, *e.g.*, erythropoietin and vascular endothelial growth factor.<sup>31</sup> Although the autoregulatory system of HIF-1 $\alpha$  has not been fully elucidated, there appears to be a negative feedback system.<sup>32</sup> *In vitro*, hypoxia induces HIF-1 $\alpha$  expression in a dose-dependent fashion, but prolonged hypoxia results in down-regulation of HIF-1 $\alpha$ , mediated by a micro-RNA, which targets HIF (aHIF), of which levels increase over time under hypoxic conditions.<sup>33</sup> In contrast to *in vitro* data, where hypoxia has only been shown to prevent HIF-1 $\alpha$  degradation, hypoxia *in vivo* stimulates transcription of HIF-1 $\alpha$ , followed by a decrease to baseline levels, possibly resulting from the aHIF-mediated negative feedback.<sup>34</sup> Human studies revealed a large interindividual variability in leukocyte HIF-1 $\alpha$  expression<sup>35</sup> and downstream target gene expression<sup>36</sup> in response to hypoxia, implicating phenotypical differences in HIF regulation.

### Involvement of HIF-1 $\alpha$ and Adenosine Signaling in the Immunologic Effects of Hypoxia

#### *The Molecular Interplay among Hypoxia, HIFs, and NF- $\kappa$ B*

The regulation of HIF-1 $\alpha$  and NF- $\kappa$ B, the latter considered the master regulator of inflammatory responses, is highly intertwined.<sup>37,38</sup> In the inactivated state, NF- $\kappa$ B is bound to the inhibitory protein I $\kappa$ B $\alpha$  in the cytosol. Not only inflammatory stimuli but also other signals, activate the enzyme I $\kappa$ B kinase (IKK), resulting in phosphorylation of I $\kappa$ B $\alpha$ . Subsequently, NF- $\kappa$ B translocates into the nucleus, and an inflammatory response characterized by production of inflammatory cytokines is generated.<sup>39</sup> Another downstream effect of NF- $\kappa$ B activity is enhanced HIF-1 $\alpha$  transcription.<sup>40-42</sup> Conversely, HIF-1 $\alpha$  activity enhances NF- $\kappa$ B activity by increasing abundance of IKK and the NF- $\kappa$ B subunit p65.<sup>41,43</sup> Moreover, hypoxia prevents PHD-dependent IKK degradation.<sup>44</sup> *In vitro* studies confirmed this effect, as combined inhibition of PHD-1 and FIH enhanced basal NF- $\kappa$ B activity in a HIF-1 $\alpha$ -independent fashion.<sup>45</sup> Paradoxically, PHD-1 and FIH inhibition suppress NF- $\kappa$ B activity under inflammatory conditions.<sup>45</sup> These data illustrate that there is extensive interplay between hypoxia, oxygen-dependent hydroxylases, HIF-1 $\alpha$ , and NF- $\kappa$ B. Furthermore,



**Fig. 1.** Hypoxia-inducible factor (HIF)-1 $\alpha$  regulation and signaling under normoxic, hypoxic, and inflammatory conditions. HIF-1 $\alpha$  subunits are constantly produced but rapidly degraded under normoxic conditions. Several pathways of HIF-1 $\alpha$  regulation have been described. First, under normoxic conditions, HIF-1 $\alpha$  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 HIF $\beta$ . During oxygen deficiency, PHD and FIH activities decrease, resulting in accumulation of HIF-1 $\alpha$  subunits in the cytosol. The receptor for activated C kinase 1 (RACK1) and heat shock protein 90 (HSP90) regulate HIF-1 $\alpha$  in an oxygen-independent manner: RACK1 facilitates oxygen-independent proteasomal degradation of HIF-1 $\alpha$ , while HSP90 competes with RACK1, thereby stabilizing HIF-1 $\alpha$ , and facilitates its transactivation. Upon accumulation, HIF-1 $\alpha$  is coactivated by P300/CBP and dimerizes with HIF $\beta$  to form stable HIF-1 $\alpha\beta$  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 $\alpha$  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-1 $\alpha$  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-1 $\alpha$  accumulation. NF- $\kappa$ B = nuclear factor of kappa-light-chain-enhancer of activated B cells; TLR = toll-like receptor.

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

#### Cellular and In Vivo Immunologic Effects of HIF-1 $\alpha$

At the cellular level, HIF-1 $\alpha$  stabilization in immune cells results in a differentiated response, highly depending on the cell type. In neutrophils, the induction of  $\beta_2$ -integrin involved in epithelial neutrophil binding,<sup>16</sup> regulation of pathogen-binding neutrophil extracellular traps, and anti-bacterial activity<sup>46</sup> are all HIF-1 $\alpha$  dependent.<sup>46</sup> HIF-1 $\alpha$

stabilization inhibits apoptosis of macrophages and neutrophils<sup>43,47</sup> and is involved in the differentiation of monocytes to macrophages as well as in macrophage maturation.<sup>48</sup> HIF-1 $\alpha$  also results in increased expression of toll-like receptor 4<sup>49</sup> as well as in enhanced macrophage phagocytosis<sup>50</sup> and bacterial killing.<sup>51</sup>

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

streptococcal skin infections than their wild-type littermates, which indicates that HIF-1 $\alpha$  in myeloid cells is essential to mount an inflammatory response required to clear local infection.<sup>51</sup> In severe systemic inflammation induced by lipopolysaccharide (to mimic Gram-negative infection)<sup>52</sup> or lipoteichoic acid and peptidoglycan (to mimic Gram-positive infection),<sup>53</sup> myeloid HIF-1 $\alpha$ -deficient mice display an attenuated inflammatory response, associated with less tissue damage and improved survival.<sup>52</sup> In accordance, HIF-1 $\alpha$  gain of function results in an overwhelming inflammatory response in sterile and bacterial peritonitis, with aggravated organ damage and impaired survival.<sup>1</sup> As such, in myeloid cells, HIF-1 $\alpha$  is essential for the generation of an effective inflammatory response to clear infections, while simultaneously, HIF-1 $\alpha$  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-1 $\alpha$  activity induces antiinflammatory and tissue-protective effects in lymphocytes. For instance, HIF-1 $\alpha$  induction results in increased numbers of regulatory T cells, with subsequent tissue protection due to attenuation of inflammation.<sup>54</sup> Furthermore, in a murine bacterial peritonitis model, T-cell-specific HIF-1 $\alpha$  deficiency results in increased levels of proinflammatory cytokines.<sup>55</sup> Suggestive of antiinflammatory effects of HIF-1 $\alpha$  in B cells, PHD inhibition with dimethylloxylglycine before lipopolysaccharide administration in mice resulted in enhanced interleukin-10 production by B1 cells, which skewed macrophages toward an antiinflammatory M2-like phenotype.<sup>56</sup> Moreover, other studies demonstrate that the transcriptional program that drives antiinflammatory regulatory T-cell differentiation is under the control of HIF *via* the induction of the HIF-target gene *FoxP3*.<sup>54</sup>

Apart from effects in dedicated immune cells, HIF-1 $\alpha$  stabilization also exerts immunologic effects in other cells, *e.g.*, intestinal and alveolar epithelium and myocytes. Pharmacologic stabilization of HIF-1 $\alpha$  through PHD inhibition in murine chemical-induced colitis results in reduced levels of TNF $\alpha$ , interleukin-6, and interleukin-1 $\beta$ , while levels of antiinflammatory interleukin-10 increase<sup>57</sup> and clinical outcome improves.<sup>58,59</sup> Similarly, pharmacologic PHD inhibition in ventilator-induced lung injury results in HIF-1 $\alpha$ -dependent reduced lung injury and prolonged survival, whereas HIF-1 $\alpha$  inhibition aggravates lung injury and shortened survival.<sup>4</sup> The tissue-protective effects of HIF-1 $\alpha$  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-1 $\alpha$ ,<sup>60,61</sup> and myocardial HIF-1 $\alpha$  expression mediates a metabolic switch to glycolysis, which is crucial for adaptation to ischemia.<sup>62</sup> An overview of the immunologic effects of PHD inhibition in *in vitro* and animal studies is provided in table 2.<sup>45,63-74</sup>

Altogether, HIF-1 $\alpha$  activity in myeloid cells is involved in the orchestration of immune responses aimed at pathogen clearance, whereas HIF-1 $\alpha$  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-1 $\alpha$  activity suppresses T-cell responses.<sup>75</sup> 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.<sup>76</sup>

### HIF-1 $\alpha$ in Sepsis

The role of HIF-1 $\alpha$  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.<sup>77</sup>

Based on the data described earlier, HIF-1 $\alpha$  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.<sup>20</sup> However, this single animal study does not fully reflect the complex dynamics of HIF-1 $\alpha$  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.<sup>78,79</sup> Fortunately, two recent observational studies in sepsis patients have increased our understanding of the dynamics of HIF-1 $\alpha$  during sepsis. In one of these, samples were obtained within 2 to 4 h after admission, and HIF-1 $\alpha$  mRNA expression in monocytes was increased.<sup>80</sup> Furthermore, HIF-1 $\alpha$  induced the negative toll-like receptor regulator interleukin-1 receptor-associated kinase M, resulting in immunosuppression.<sup>80</sup> In contrast, the other study found reduced leukocytic HIF-1 $\alpha$  protein and mRNA expression, but samples were obtained at later time points (*i.e.*, within 24 h after admission).<sup>81</sup> 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-1 $\alpha$  expression, resulting in the induction of negative regulators such as interleukin-1 receptor-associated kinase M to

**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>	63	Microglial cell line (BV2)	Lipopolysaccharide	EDHB	↓ mRNA, TNF $\alpha$ , and interleukin-6
	64	Keratinocyte cell line (HaCaT)	Lipopolysaccharide	AKB-4924	↑ VEGF, interleukin-6, interleukin-8
	65	Monocyte/macrophage cell line (U937) and neutrophils from healthy donors	Various Gram-positive and negative bacteria	AKB-4924	↑ Bactericidal activity
	66	Endothelial cell line (5A32)	TNF $\alpha$	Dimethyloxalyglycine	↓ VCAM-1
	67	Macrophage cell line (RAW264.7)	Lipopolysaccharide	Dimethyloxalyglycine	↓ TNF $\alpha$
	45	HeLa cell line	Interleukin-1 $\beta$	Dimethyloxalyglycine	↓ NF- $\kappa$ B activity
	Animal model	58	Mice	TNBS (chemical colitis)	FG-4497
59		Mice	DSS (chemical colitis)	Dimethyloxalyglycine	↓ Colonic interleukin-1 $\beta$ , TNF $\alpha$ , interleukin-12, interleukin-6, disease activity index, weight loss, histologic inflammation
74		Rats	DNBS (chemical colitis)	Dimethyloxalyglycine	↓ Neutrophil infiltration
68		Mice	TNBS and DSS (chemical colitis)	TRC160334	↓ Disease activity index, weight loss, histologic inflammation
57		Mice	TNBS (chemical colitis)	AKB-4924	↓ Serum interleukin-1 $\beta$ , TNF $\alpha$ , interleukin-6, weight loss, disease activity ↑ Interleukin-10
69		Mice	TNBS (chemical colitis)	AKB-4924	↓ Colonic interleukin-1 $\beta$ , TNF $\alpha$ , interleukin-12, interleukin-6, weight loss, histologic inflammation
70		Mice	TNF <sup>AARE/+</sup> mice (spontaneous chronic terminal ileitis)	Dimethyloxalyglycine	↓ Histologic inflammation
71		Mice	Lipopolysaccharide intraperitoneal (endotoxemic shock)	Dimethyloxalyglycine	↓ TNF $\alpha$ , mortality ↑ Interleukin-10
4		Mice	Ventilator-induced lung injury	Dimethyloxalyglycine	↓ BAL MPO, pulmonary edema ↑ Gas exchange, survival time
65		Mice	<i>Staphylococcus aureus</i> (cutaneous infection)	AKB-4924	↓ Lesion size, bacterial load, disease severity
72		Mice	<i>Escherichia coli</i> (urinary tract infection)	AKB-4924	↓ Bacterial load, interleukin-1 $\beta$ , interleukin-6, KC, myeloperoxidase activity
63		Mice	MPTP (neurotoxicity)	EDHB	↓ Striatal interleukin-6
73		Rabbits	Lipopolysaccharide and methylprednisolone (osteonecrosis)	EDHB	↓ Osteonecrosis

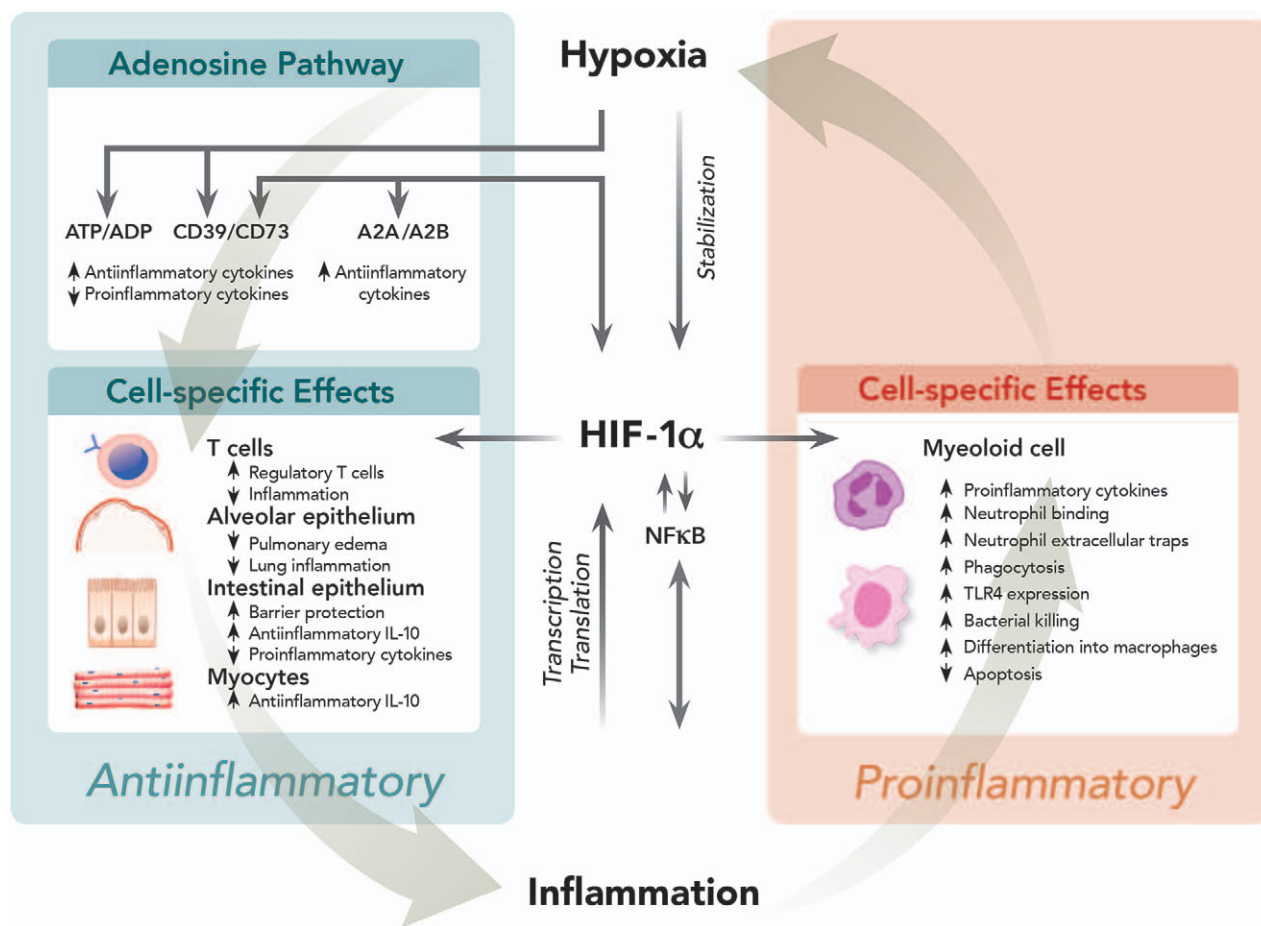
BAL = bronchial alveolar lavage; DNBS = dinitrobenzene sulfonic acid; DSS = dextran sulfate sodium; EDHB = ethyl-3,4-dihydroxybenzoate; KC = keratinocyte-derived chemokine; MPO = myeloperoxidase; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NF- $\kappa$ B = nuclear factor of kappa-light-chain-enhancer of activated B cells; PHD = prolyl hydroxylase; TNBS = 2,4,6-trinitrobenzene sulfonic acid; TNF $\alpha$  = tumor necrosis factor alpha; TNF<sup>AARE/+</sup> mice = mice with gene targeted alterations in untranslated region of TNF $\alpha$  mRNA leading to development of severe ileitis; VCAM-1 = vascular cell adhesion molecule-1; VEGF = vascular endothelial growth factor.

counteract excessive inflammation, ultimately resulting in reduced HIF-1 $\alpha$  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-1 $\alpha$  dependent.<sup>82,83</sup>

Cellular distress (*e.g.*, hypoxia<sup>84</sup>) results in increased availability of the adenosine progenitors adenosine triphosphate and adenosine diphosphate.<sup>85</sup> Hypoxia leads to up-regulation of CD39 (ectoapyrase),<sup>86,87</sup> which converts adenosine triphosphate and adenosine diphosphate into adenosine monophosphate, and to HIF-1 $\alpha$ -dependent up-regulation of CD73 (5'-ectonucleotidase), which converts adenosine monophosphate into adenosine.<sup>82</sup> The tissue-protective



**Fig. 2.** The interaction between hypoxia and inflammation. Hypoxia enhances the immune response and is an inflammatory stimulus by itself. Hypoxia leads to cellular stabilization of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ), resulting in a synergistic effect with the key inflammatory transcription factor nuclear factor of kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B). In addition, inflammation enhances transcription and translation of HIF-1 $\alpha$ , leading to a synergistic effect in case of hypoxia and inflammation. In myeloid cells, such as neutrophils and monocytes, HIF-1 $\alpha$  activity exerts proinflammatory effects, aimed at clearance of pathogens. Conversely, in many other cells, such as T cells, pulmonary and interstitial epithelium, and myocytes, HIF-1 $\alpha$  activity has antiinflammatory effects. Furthermore, hypoxia exerts antiinflammatory 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 antiinflammatory adenosine 2A and 2B receptors (A2A and A2B). The up-regulation of CD73 and adenosine receptors is HIF-1 $\alpha$  dependent. IL-10 = interleukin-10; TLR4 = toll-like receptor 4.

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 injury.<sup>88–90</sup> Correspondingly, genetic overexpression of HIF-1 $\alpha$  results in increased epithelial expression of CD73 and improves outcome in murine chemically induced colitis.<sup>91</sup> Finally, hypoxia increases the expression of the adenosine 2A (A2A) and 2B (A2B) receptors, the latter in a HIF-1 $\alpha$ -dependent manner.<sup>83</sup> Stimulation of these receptors results in systemic antiinflammatory effects in murine models of ischemia–reperfusion,<sup>89</sup> hypoxia,<sup>92</sup> and inflammation.<sup>93</sup> Furthermore, permissive hypoxia (fraction of inspired oxygen [F<sub>I</sub>O<sub>2</sub>], 10%) attenuated lung damage and improved survival in a murine model of acute lung injury

in an A2A receptor-dependent manner,<sup>94</sup> and induction of the A2B-receptor in type 1 alveolar cells during ventilator-induced lung injury was shown to be dependent on HIF-1 $\alpha$ .<sup>3</sup> Similarly, hypoxic preconditioning protected mice from liver ischemia and reperfusion injury in an A2B receptor-dependent manner.<sup>95</sup>

The limited human data available substantiate that hypoxia results in enhanced adenosine availability. For instance, exposure to short-term hypoxia (20 min; Sao<sub>2</sub>, 80%) in healthy volunteers increases plasma adenosine levels.<sup>96</sup> Furthermore, several experimental human studies have demonstrated antiinflammatory effects of adenosine signaling, as intravenous adenosine administration<sup>97</sup> as well as oral treatment with the adenosine uptake inhibitor

dipyridamole<sup>98</sup> attenuated the proinflammatory interleukin-6 response during experimental human endotoxemia, and dipyridamole treatment also augmented antiinflammatory interleukin-10 production.<sup>98</sup> However, increased adenosine availability in these latter studies was not induced by hypoxia. Finally, a proof-of-concept clinical study revealed that interferon- $\beta$ -1a enhances CD73 expression in human lung tissue and that administration of this cytokine to acute respiratory distress syndrome (ARDS) patients is associated with reduced interleukin-6 and interleukin-8 levels as well as improved PaO<sub>2</sub>/F<sub>IO</sub><sub>2</sub> ratios and survival.<sup>99</sup>

In addition to HIF-1 $\alpha$ , NF- $\kappa$ 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 in Ref. <sup>100</sup>).

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 to 33%,<sup>101,102</sup> depending on the definition (usually the need for mechanical ventilation and/or a PaO<sub>2</sub>/F<sub>IO</sub><sub>2</sub> ratio of less than 300 mmHg<sup>101-103</sup>), and is associated with a mortality of 31 to 52%.<sup>101-103</sup> A subcategory of hypoxic respiratory failure is ARDS, comprising 3 to 70% of patients with respiratory failure.<sup>101-103</sup> 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%.<sup>103-109</sup> 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 PaO<sub>2</sub>), as patients with hypoxic respiratory failure can have normal PaO<sub>2</sub> levels. The occurrence of hypoxia (*i.e.*, PaO<sub>2</sub> less than 80 mmHg) at ICU admission is frequent (40%),<sup>110</sup> 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.<sup>110</sup> The association between hypoxia at ICU admission and increased mortality was confirmed in a similar analysis in Australian and New Zealand ICU patients.<sup>111</sup> 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 SaO<sub>2</sub> 88 to 95% is used in ARDS studies,<sup>112</sup> even though there is no solid evidence supporting these targets.<sup>113</sup>

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 O<sub>2</sub>-ICU study randomizes ICU patients with systemic

inflammation to either a target PaO<sub>2</sub> 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  $\times$  2 fashion to normoxia (SaO<sub>2</sub>, 88 to 95%) versus F<sub>IO</sub><sub>2</sub> 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.<sup>114</sup> 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.<sup>115</sup> 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 SaO<sub>2</sub> targets of 90 to 92%<sup>116</sup> and 92 to 95%,<sup>117</sup> 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 (SpO<sub>2</sub>, greater than 96%) with a conservative strategy (SpO<sub>2</sub>, 88 to 92%).<sup>118</sup> 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.<sup>119</sup> As many specific therapeutic target interventions have failed to show benefit in clinical trials,<sup>120</sup> it would be naive 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 hyperoxia or even permitting or inducing hypoxia. For example, animal data suggest that averting hyperoxia and even permitting hypoxia is beneficial in acute lung injury,<sup>94</sup> 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  $P_{aO_2}$  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<sup>121</sup> 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.*,  $S_{aO_2}$ , less than 90%).<sup>122</sup> 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 $\alpha$ -mediated effects could also be pursued through pharmacologic inhibition of PHDs. The PHD inhibitor FG-4497 increased HIF-1 $\alpha$  stabilization in mice, with subsequent resistance of stem cells to irradiation,<sup>123</sup> improved kidney transplantation survival,<sup>124</sup> and attenuated TNF $\alpha$  expression and weight loss during colitis.<sup>58</sup> A comparable PHD inhibitor (FG-2216) resulted in increased plasma erythropoietin levels in hemodialysis patients<sup>125</sup>; 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.<sup>126</sup> Nonetheless, clinical trials with new drugs targeting PHDs for treatment of anemia in patients with chronic renal disease and dialysis are currently being performed.<sup>26</sup> Whether pharmacologic HIF stimulation affects the immune response in humans has not been established yet. Additionally, the frequently used PHD inhibitor dimethyloxalylglycine and the aforementioned FG compounds are pan-hydroxylase inhibitors and are not specific for HIF-1 $\alpha$  stabilization, which may lead to undesired effects. For example, dimethyloxalylglycine also stabilizes HIF-2 $\alpha$ , which results in increased erythropoietin levels<sup>58,59</sup> 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.<sup>69</sup>

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, antiinflammatory strategies in sepsis have been unsuccessful in the past,<sup>120</sup> 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.<sup>77</sup> 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.<sup>127</sup>

## Conclusion

There is extensive interplay between hypoxia and the immune system. Hypoxia and inflammation synergistically induce HIF-1 $\alpha$  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-1 $\alpha$  stabilizers and agents acting on the adenosine pathway are required. In any case, the optimal  $P_{aO_2}$  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, POI-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.

## Correspondence

Address correspondence to Dr. Kox: Department of Intensive Care Medicine (710), Radboud University Medical Centre, Geert Grooteplein Zuid 10, PO Box 9101, 6500 HB Nijmegen, The Netherlands. matthijs.kox@radboudumc.nl. Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

## References

- Kiss J, Mollenhauer M, Walmsley SR, Kirchberg J, Radhakrishnan P, Niemietz T, Dudda J, Steinert G, Whyte MK, Carmeliet P, Mazzone M, Weitz J, Schneider M: Loss of the oxygen sensor PHD3 enhances the innate immune response to abdominal sepsis. *J Immunol* 2012; 189:1955–65
- Nizet V, Johnson RS: Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol* 2009; 9:609–17
- Eckle T, Kewley EM, Brodsky KS, Tak E, Bonney S, Gobel M, Anderson D, Glover LE, Riegel AK, Colgan SP, Eltzschig HK: Identification of hypoxia-inducible factor HIF-1A as transcriptional regulator of the A2B adenosine receptor during acute lung injury. *J Immunol* 2014; 192:1249–56
- Eckle T, Brodsky K, Bonney M, Packard T, Han J, Borchers CH, Mariani TJ, Kominsky DJ, Mittelbronn M, Eltzschig HK: HIF1A reduces acute lung injury by optimizing carbohydrate metabolism in the alveolar epithelium. *PLoS Biol* 2013; 11:e1001665
- Bartels K, Grenz A, Eltzschig HK: Hypoxia and inflammation are two sides of the same coin. *Proc Natl Acad Sci USA* 2013; 110:18351–2
- Fröhlich S, Boylan J, McLoughlin P: Hypoxia-induced inflammation in the lung: A potential therapeutic target in acute lung injury? *Am J Respir Cell Mol Biol* 2013; 48:271–9
- Eltzschig HK, Carmeliet P: Hypoxia and inflammation. *N Engl J Med* 2011; 364:656–65
- Semenza GL: Hypoxia-inducible factors in physiology and medicine. *Cell* 2012; 148:399–408
- Semenza GL: Oxygen sensing, homeostasis, and disease. *N Engl J Med* 2011; 365:537–47
- Kuhlicke J, Frick JS, Morote-Garcia JC, Rosenberger P, Eltzschig HK: Hypoxia inducible factor (HIF)-1 coordinates induction of Toll-like receptors TLR2 and TLR6 during hypoxia. *PLoS One* 2007; 2:e1364
- Querido JS, Sheel AW, Cheema R, Van Eeden S, Mulgrew AT, Ayas NT: Effects of 10 days of modest intermittent hypoxia on circulating measures of inflammation in healthy humans. *Sleep Breath* 2012; 16:657–62
- Burki NK, Tetenta SU: Inflammatory response to acute hypoxia in humans. *Pulm Pharmacol Ther* 2013; 8–11
- Ghezzi P, Dinarello CA, Bianchi M, Rosandich ME, Repine JE, White CW: Hypoxia increases production of interleukin-1 and tumor necrosis factor by human mononuclear cells. *Cytokine* 1991; 3:189–94
- Scannell G, Waxman K, Kaml GJ, Ioli G, Gatanaga T, Yamamoto R, Granger GA: Hypoxia induces a human macrophage cell line to release tumor necrosis factor-alpha and its soluble receptors *in vitro*. *J Surg Res* 1993; 54:281–5
- Naldini A, Carraro F, Silvestri S, Bocci V: Hypoxia affects cytokine production and proliferative responses by human peripheral mononuclear cells. *J Cell Physiol* 1997; 173:335–42
- Kong T, Eltzschig HK, Karhausen J, Colgan SP, Shelley CS: Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of beta2 integrin gene expression. *Proc Natl Acad Sci USA* 2004; 101:10440–5
- Jantsch J, Chakravorty D, Turza N, Prectel AT, Buchholz B, Gerlach RG, Volke M, Gläsner J, Warnecke C, Wiesener MS, Eckardt KU, Steinkasserer A, Hensel M, Willam C: Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. *J Immunol* 2008; 180:4697–705
- Matuschak GM, Nayak R, Doyle TM, Lechner AJ: Acute hypoxia decreases *E. coli* LPS-induced cytokine production and NF-kappaB activation in alveolar macrophages. *Respir Physiol Neurobiol* 2010; 172:63–71
- Rahat MA, Bitterman H, Lahat N: Molecular mechanisms regulating macrophage response to hypoxia. *Front Immunol* 2011; 2:45
- Baze MM, Hunter K, Hayes JP: Chronic hypoxia stimulates an enhanced response to immune challenge without evidence of an energetic tradeoff. *J Exp Biol* 2011; 214(pt 19):3255–68
- Klausen T, Olsen NV, Poulsen TD, Richalet JP, Pedersen BK: Hypoxemia increases serum interleukin-6 in humans. *Eur J Appl Physiol Occup Physiol* 1997; 76:480–2
- Hartmann G, Tschöp M, Fischer R, Bidlingmaier C, Riepl R, Tschöp K, Hautmann H, Endres S, Toepfer M: High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine* 2000; 12:246–52
- Hitomi Y, Miyamura M, Mori S, Suzuki K, Kizaki T, Itoh C, Murakami K, Haga S, Ohno H: Intermittent hypobaric hypoxia increases the ability of neutrophils to generate superoxide anion in humans. *Clin Exp Pharmacol Physiol* 2003; 30:659–64
- Wang JS, Liu HC: Systemic hypoxia enhances bactericidal activities of human polymorphonuclear leucocytes. *Clin Sci (Lond)* 2009; 116:805–17
- Fritzenwanger M, Jung C, Goebel B, Lauten A, Figulla HR: Impact of short-term systemic hypoxia on phagocytosis, cytokine production, and transcription factor activation in peripheral blood cells. *Mediators Inflamm* 2011; 2011:429501
- Eltzschig HK, Bratton DL, Colgan SP: Targeting hypoxia signalling for the treatment of ischaemic and inflammatory diseases. *Nat Rev Drug Discov* 2014; 13:852–69
- Minet E, Mottet D, Michel G, Roland I, Raes M, Remacle J, Michiels C: Hypoxia-induced activation of HIF-1: Role of HIF-1alpha-Hsp90 interaction. *FEBS Lett* 1999; 460:251–6
- Masoud GN, Li W: HIF-1α pathway: Role, regulation and intervention for cancer therapy. *Acta Pharm Sin B* 2015; 5:378–89
- Liu YV, Baek JH, Zhang H, Diez R, Cole RN, Semenza GL: RACK1 competes with HSP90 for binding to HIF-1alpha and is required for O(2)-independent and HSP90 inhibitor-induced degradation of HIF-1alpha. *Mol Cell* 2007; 25:207–17
- Ke Q, Costa M: Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 2006; 70:1469–80
- Hellwig-Bürgel T, Rutkowski K, Metzén E, Fandrey J, Jelkmann W: Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. *Blood* 1999; 94:1561–7
- Bruning U, Cerone L, Neufeld Z, Fitzpatrick SF, Cheong A, Scholz CC, Simpson DA, Leonard MO, Tambuwala MM, Cummins EP, Taylor CT: MicroRNA-155 promotes resolution of hypoxia-inducible factor 1alpha activity during prolonged hypoxia. *Mol Cell Biol* 2011; 31:4087–96
- Poitz DM, Augstein A, Hesse K, Christoph M, Ibrahim K, Braun-Dullaeus RC, Strasser RH, Schmeißer A: Regulation of the HIF-system in human macrophages—Differential regulation of HIF-α subunits under sustained hypoxia. *Mol Immunol* 2014; 57:226–35
- Pialoux V, Mounier R, Brown AD, Steinback CD, Rawling JM, Poulin MJ: Relationship between oxidative stress and HIF-1 alpha mRNA during sustained hypoxia in humans. *Free Radic Biol Med* 2009; 46:321–6

35. Tissot van Patot MC, Serkova NJ, Haschke M, Kominsky DJ, Roach RC, Christians U, Henthorn TK, Honigman B: Enhanced leukocyte HIF-1 $\alpha$  and HIF-1 DNA binding in humans after rapid ascent to 4300 m. *Free Radic Biol Med* 2009; 46:1551–7
36. Brooks JT, Elvidge GP, Glennly L, Gleadle JM, Liu C, Ragoussis J, Smith TG, Talbot NP, Winchester L, Maxwell PH, Robbins PA: Variations within oxygen-regulated gene expression in humans. *J Appl Physiol* (1985) 2009; 106:212–20
37. Frede S, Stockmann C, Freitag P, Fandrey J: Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes *via* p44/42 MAPK and NF-kappaB. *Biochem J* 2006; 396:517–27
38. Taylor CT: Interdependent roles for hypoxia inducible factor and nuclear factor-kappaB in hypoxic inflammation. *J Physiol* 2008; 586:4055–9
39. Taylor CT, Cummins EP: The role of NF-kappaB in hypoxia-induced gene expression. *Ann N Y Acad Sci* 2009; 1177:178–84
40. Bonello S, Zähringer C, BelAiba RS, Djordjevic T, Hess J, Michiels C, Kietzmann T, Görlach A: Reactive oxygen species activate the HIF-1 $\alpha$  promoter *via* a functional NFkappaB site. *Arterioscler Thromb Vasc Biol* 2007; 27:755–61
41. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG, Karin M: NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1 $\alpha$ . *Nature* 2008; 453:807–11
42. Belaiba RS, Bonello S, Zähringer C, Schmidt S, Hess J, Kietzmann T, Görlach A: Hypoxia up-regulates hypoxia-inducible factor-1 $\alpha$  transcription by involving phosphatidylinositol 3-kinase and nuclear factor kappaB in pulmonary artery smooth muscle cells. *Mol Biol Cell* 2007; 18:4691–7
43. Walmsley SR, Print C, Farahi N, Peyssonnaud C, Johnson RS, Cramer T, Sobolewski A, Condliffe AM, Cowburn AS, Johnson N, Chilvers ER: Hypoxia-induced neutrophil survival is mediated by HIF-1 $\alpha$ -dependent NF-kappaB activity. *J Exp Med* 2005; 201:105–15
44. Cummins EP, Berra E, Comerford KM, Ginouves A, Fitzgerald KT, Seeballuck F, Godson C, Nielsen JE, Moynagh P, Pouyssegur J, Taylor CT: Prolyl hydroxylase-1 negatively regulates IkappaB kinase-beta, giving insight into hypoxia-induced NFkappaB activity. *Proc Natl Acad Sci USA* 2006; 103:18154–9
45. Scholz CC, Cavadas MA, Tambuwala MM, Hams E, Rodríguez J, von Kriegsheim A, Cotter P, Bruning U, Fallon PG, Cheong A, Cummins EP, Taylor CT: Regulation of IL-1 $\beta$ -induced NF- $\kappa$ B by hydroxylases links key hypoxic and inflammatory signaling pathways. *Proc Natl Acad Sci USA* 2013; 110:18490–5
46. McInturff AM, Cody MJ, Elliott EA, Glenn JW, Rowley JW, Rondina MT, Yost CC: Mammalian target of rapamycin regulates neutrophil extracellular trap formation *via* induction of hypoxia-inducible factor 1  $\alpha$ . *Blood* 2012; 120:3118–25
47. McGovern NN, Cowburn AS, Porter L, Walmsley SR, Summers C, Thompson AA, Anwar S, Willcocks LC, Whyte MK, Condliffe AM, Chilvers ER: Hypoxia selectively inhibits respiratory burst activity and killing of *Staphylococcus aureus* in human neutrophils. *J Immunol* 2011; 186:453–63
48. Oda T, Hirota K, Nishi K, Takabuchi S, Oda S, Yamada H, Arai T, Fukuda K, Kita T, Adachi T, Semenza GL, Nohara R: Activation of hypoxia-inducible factor 1 during macrophage differentiation. *Am J Physiol Cell Physiol* 2006; 291:C104–13
49. Kim SY, Choi YJ, Joung SM, Lee BH, Jung YS, Lee JY: Hypoxic stress up-regulates the expression of toll-like receptor 4 in macrophages *via* hypoxia-inducible factor. *Immunology* 2010; 129:516–24
50. Anand RJ, Gripar SC, Li J, Kohler JW, Branca MF, Dubowski T, Sodhi CP, Hackam DJ: Hypoxia causes an increase in phagocytosis by macrophages in a HIF-1 $\alpha$ -dependent manner. *J Leukoc Biol* 2007; 82:1257–65
51. Peyssonnaud C, Datta V, Cramer T, Doedens A, Theodorakis EA, Gallo RL, Hurtado-Ziola N, Nizet V, Johnson RS: HIF-1 $\alpha$  expression regulates the bactericidal capacity of phagocytes. *J Clin Invest* 2005; 115:1806–15
52. Peyssonnaud C, Cejudo-Martin P, Doedens A, Zinkernagel AS, Johnson RS, Nizet V: Cutting edge: Essential role of hypoxia inducible factor-1 $\alpha$  in development of lipopolysaccharide-induced sepsis. *J Immunol* 2007; 178:7516–9
53. Mahabeleshwar GH, Qureshi MA, Takami Y, Sharma N, Lingrel JB, Jain MK: A myeloid hypoxia-inducible factor 1 $\alpha$ -Krüppel-like factor 2 pathway regulates gram-positive endotoxin-mediated sepsis. *J Biol Chem* 2012; 287:1448–57
54. Clambey ET, McNamee EN, Westrich JA, Glover LE, Campbell EL, Jedlicka P, de Zoeten EF, Cambier JC, Stenmark KR, Colgan SP, Eltzschig HK: Hypoxia-inducible factor-1  $\alpha$ -dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc Natl Acad Sci USA* 2012; 109:E2784–93
55. Thiel M, Caldwell CC, Kreth S, Kuboki S, Chen P, Smith P, Ohta A, Lentsch AB, Lukashev D, Sitkovsky MV: Targeted deletion of HIF-1 $\alpha$  gene in T cells prevents their inhibition in hypoxic inflamed tissues and improves septic mice survival. *PLoS One* 2007; 2:e853
56. Hams E, Saunders SP, Cummins EP, O'Connor A, Tambuwala MT, Gallagher WM, Byrne A, Campos-Torres A, Moynagh PM, Jobin C, Taylor CT, Fallon PG: The hydroxylase inhibitor dimethylallyl glycine attenuates endotoxic shock *via* alternative activation of macrophages and IL-10 production by B1 cells. *Shock* 2011; 36:295–302
57. Keely S, Campbell EL, Baird AW, Hansbro PM, Shalwitz RA, Kotsakis A, McNamee EN, Eltzschig HK, Kominsky DJ, Colgan SP: Contribution of epithelial innate immunity to systemic protection afforded by prolyl hydroxylase inhibition in murine colitis. *Mucosal Immunol* 2014; 7:114–23
58. Robinson A, Keely S, Karhausen J, Gerich ME, Furuta GT, Colgan SP: Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. *Gastroenterology* 2008; 134:145–55
59. Cummins EP, Seeballuck F, Keely SJ, Mangan NE, Callanan JJ, Fallon PG, Taylor CT: The hydroxylase inhibitor dimethylallylglycine is protective in a murine model of colitis. *Gastroenterology* 2008; 134:156–65
60. Eckle T, Köhler D, Lehmann R, El Kasmi K, Eltzschig HK: Hypoxia-inducible factor-1 is central to cardioprotection: A new paradigm for ischemic preconditioning. *Circulation* 2008; 118:166–75
61. Cai Z, Luo W, Zhan H, Semenza GL: Hypoxia-inducible factor 1 is required for remote ischemic preconditioning of the heart. *Proc Natl Acad Sci USA* 2013; 110:17462–7
62. Eckle T, Hartmann K, Bonney S, Reithel S, Mittelbronn M, Walker LA, Lowes BD, Han J, Borchers CH, Buttrick PM, Kominsky DJ, Colgan SP, Eltzschig HK: Adora2b-elicited Per2 stabilization promotes a HIF-dependent metabolic switch crucial for myocardial adaptation to ischemia. *Nat Med* 2012; 18:774–82
63. Chinta SJ, Rajagopalan S, Ganesan A, Andersen JK: A possible novel anti-inflammatory mechanism for the pharmacological prolyl hydroxylase inhibitor 3,4-dihydroxybenzoate: Implications for use as a therapeutic for Parkinson's disease. *Parkinsons Dis* 2012; 2012:364684
64. Leire E, Olson J, Isaacs H, Nizet V, Hollands A: Role of hypoxia inducible factor-1 in keratinocyte inflammatory response and neutrophil recruitment. *J Inflamm (Lond)* 2013; 10:28
65. Okumura CY, Hollands A, Tran DN, Olson J, Dahesh S, von Köckritz-Blickwede M, Thienphrapa W, Corle C, Jeung SN, Kotsakis A, Shalwitz RA, Johnson RS, Nizet V: A new pharmacological agent (AKB-4924) stabilizes hypoxia inducible factor-1 (HIF-1) and increases skin innate defenses against bacterial infection. *J Mol Med (Berl)* 2012; 90:1079–89

66. Cartee TV, White KJ, Newton-West M, Swerlick RA: Hypoxia and hypoxia mimetics inhibit TNF-dependent VCAM1 induction in the 5A32 endothelial cell line *via* a hypoxia inducible factor dependent mechanism. *J Dermatol Sci* 2012; 65:86–94
67. Takeda K, Ichiki T, Narabayashi E, Inanaga K, Miyazaki R, Hashimoto T, Matsuura H, Ikeda J, Miyata T, Sunagawa K: Inhibition of prolyl hydroxylase domain-containing protein suppressed lipopolysaccharide-induced TNF- $\alpha$  expression. *Arterioscler Thromb Vasc Biol* 2009; 29:2132–7
68. Gupta R, Chaudhary AR, Shah BN, Jadhav AV, Zambad SP, Gupta RC, Deshpande S, Chauthaiwale V, Dutt C: Therapeutic treatment with a novel hypoxia-inducible factor hydroxylase inhibitor (TRC160334) ameliorates murine colitis. *Clin Exp Gastroenterol* 2014; 7:13–23
69. Marks E, Goggins BJ, Cardona J, Cole S, Minahan K, Mateer S, Walker MM, Shalwitz R, Keely S: Oral delivery of prolyl hydroxylase inhibitor: AKB-4924 promotes localized mucosal healing in a mouse model of colitis. *Inflamm Bowel Dis* 2015; 21:267–75
70. Hindryckx P, De Vos M, Jacques P, Ferdinande L, Peeters H, Olivier K, Bogaert S, Brinkman B, Vandenabeele P, Elewaut D, Laukens D: Hydroxylase inhibition abrogates TNF- $\alpha$ -induced intestinal epithelial damage by hypoxia-inducible factor-1-dependent repression of FADD. *J Immunol* 2010; 185:6306–16
71. Hams E, Saunders SP, Cummins EP, O'Connor A, Tambuwala MT, Gallagher WM, Byrne A, Campos-Torres A, Moynagh PM, Jobin C, Taylor CT, Fallon PG: The hydroxylase inhibitor dimethylallyl glycine attenuates endotoxin shock *via* alternative activation of macrophages and IL-10 production by B1 cells. *Shock* 2011; 36:295–302
72. Lin AE, Beasley FC, Olson J, Keller N, Shalwitz RA, Hannan TJ, Hultgren SJ, Nizet V: Role of hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) in innate defense against uropathogenic *Escherichia coli* infection. *PLoS Pathog* 2015; 11:e1004818
73. Fan L, Li J, Yu Z, Dang X, Wang K: Hypoxia-inducible factor prolyl hydroxylase inhibitor prevents steroid-associated osteonecrosis of the femoral head in rabbits by promoting angiogenesis and inhibiting apoptosis. *PLoS One* 2014; 9:e107774
74. Flannigan KL, Agbor TA, Motta JP, Ferraz JG, Wang R, Buret AG, Wallace JL: Proresolution effects of hydrogen sulfide during colitis are mediated through hypoxia-inducible factor-1 $\alpha$ . *FASEB J* 2015; 29:1591–602
75. Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, DeNardo DG, Coussens LM, Karin M, Goldrath AW, Johnson RS: Macrophage expression of hypoxia-inducible factor-1 $\alpha$  suppresses T-cell function and promotes tumor progression. *Cancer Res* 2010; 70:7465–75
76. Doedens AL, Phan AT, Stradner MH, Fujimoto JK, Nguyen JV, Yang E, Johnson RS, Goldrath AW: Hypoxia-inducible factors enhance the effector responses of CD8(+) T cells to persistent antigen. *Nat Immunol* 2013; 14:1173–82
77. Leentjens J, Kox M, van der Hoeven JG, Netea MG, Pickkers P: Immunotherapy for the adjunctive treatment of sepsis: From immunosuppression to immunostimulation. Time for a paradigm change? *Am J Respir Crit Care Med* 2013; 187:1287–93
78. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC, López CM, Honari S, Moore EE, Minei JP, Cuschieri J, Bankey PE, Johnson JL, Sperry J, Nathens AB, Billiar TR, West MA, Jeschke MG, Klein MB, Gamelli RL, Gibran NS, Brownstein BH, Miller-Graziano C, Calvano SE, Mason PH, Cobb JP, Rahme LG, Lowry SF, Maier RV, Moldawer LL, Herndon DN, Davis RW, Xiao W, Tompkins RG: Inflammation and Host Response to Injury, Large Scale Collaborative Research Program: Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 2013; 110:3507–12
79. Takao K, Miyakawa T: Genomic responses in mouse models greatly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 2015; 112:1167–72
80. Shalova IN, Lim JY, Chittezhath M, Zinkernagel AS, Beasley F, Hernández-Jiménez E, Toledano V, Cubillos-Zapata C, Rapisarda A, Chen J, Duan K, Yang H, Poidinger M, Melillo G, Nizet V, Arnalich F, López-Collazo E, Biswas SK: Human monocytes undergo functional re-programming during sepsis mediated by hypoxia-inducible factor-1 $\alpha$ . *Immunity* 2015; 42:484–98
81. Schäfer ST, Frede S, Winning S, Bick A, Roshangar P, Fandrey J, Peters J, Adamzik M: Hypoxia-inducible factor and target gene expression are decreased in patients with sepsis: Prospective observational clinical and cellular studies. *ANESTHESIOLOGY* 2013; 118:1426–36
82. Warner DS, Warner MA, Eltzschig HK: Adenosine : An old drug newly discovered. *ANESTHESIOLOGY* 2009; 111:904–15
83. Kong T, Westerman KA, Faigle M, Eltzschig HK, Colgan SP: HIF-dependent induction of adenosine A2B receptor in hypoxia. *FASEB J* 2006; 20:2242–50
84. Lim To WK, Kumar P, Marshall JM: Hypoxia is an effective stimulus for vesicular release of ATP from human umbilical vein endothelial cells. *Placenta* 2015; 36:759–66
85. Eltzschig HK, Sitkovsky MV, Robson SC: Purinergic signaling during inflammation. *N Engl J Med* 2012; 367:2322–33
86. Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjoji K, Robson SC, Colgan SP: Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: Role of ectonucleotidases and adenosine A2B receptors. *J Exp Med* 2003; 198:783–96
87. Eltzschig HK, Köhler D, Eckle T, Kong T, Robson SC, Colgan SP: Central role of Sp1-regulated CD39 in hypoxia/ischemia protection. *Blood* 2009; 113:224–32
88. Köhler D, Eckle T, Faigle M, Grenz A, Mittelbronn M, Laucher S, Hart ML, Robson SC, Müller CE, Eltzschig HK: CD39/ectonucleoside triphosphate diphosphohydrolase 1 provides myocardial protection during cardiac ischemia/reperfusion injury. *Circulation* 2007; 116:1784–94
89. Hart ML, Grenz A, Gorzolla IC, Schittenhelm J, Dalton JH, Eltzschig HK: Hypoxia-inducible factor-1 $\alpha$ -dependent protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. *J Immunol* 2011; 186:4367–74
90. Ehrentraut H, Clambey ET, McNamee EN, Brodsky KS, Ehrentraut SF, Poth JM, Riegel AK, Westrich JA, Colgan SP, Eltzschig HK: CD73+ regulatory T cells contribute to adenosine-mediated resolution of acute lung injury. *FASEB J* 2013; 27:2207–19
91. Karhausen J, Furuta GT, Tomaszewski JE, Johnson RS, Colgan SP, Haase VH: Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. *J Clin Invest* 2004; 114:1098–106
92. Eckle T, Grenz A, Laucher S, Eltzschig HK: A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. *J Clin Invest* 2008; 118:3301–15
93. Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK: A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood* 2008; 111:2024–35
94. Thiel M, Chouker A, Ohta A, Jackson E, Caldwell C, Smith P, Lukashev D, Bittmann I, Sitkovsky MV: Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. *PLoS Biol* 2005; 3:e174
95. Choukèr A, Ohta A, Martignoni A, Lukashev D, Zacharia LC, Jackson EK, Schnermann J, Ward JM, Kaufmann I, Klauenberg B, Sitkovsky MV, Thiel M: *In vivo* hypoxic preconditioning protects from warm liver ischemia-reperfusion injury through the adenosine A2B receptor. *Transplantation* 2012; 94:894–902

96. Saito H, Nishimura M, Shinano H, Makita H, Tsujino I, Shibuya E, Sato F, Miyamoto K, Kawakami Y: Plasma concentration of adenosine during normoxia and moderate hypoxia in humans. *Am J Respir Crit Care Med* 1999; 159:1014–8
97. Soop A, Johansson C, Hjemdahl P, Kristiansson M, Gyllenhammar H, Li N, Sollevi A: Adenosine treatment attenuates cytokine interleukin-6 responses to endotoxin challenge in healthy volunteers. *Shock* 2003; 19:503–7
98. Ramakers BP, Riksen NP, Stal TH, Heemskerk S, van den Broek P, Peters WH, van der Hoeven JG, Smits P, Pickkers P: Dipyridamole augments the antiinflammatory response during human endotoxemia. *Crit Care* 2011; 15:R289
99. Bellingan G, Maksimow M, Howell DC, Stotz M, Beale R, Beatty M, Walsh T, Binning A, Davidson A, Kuper M, Shah S, Cooper J, Waris M, Yegutkin GG, Jalkanen J, Salmi M, Piippo I, Jalkanen M, Montgomery H, Jalkanen S: The effect of intravenous interferon-beta-1a (FP-1201) on lung CD73 expression and on acute respiratory distress syndrome mortality: An open-label study. *Lancet Respir Med* 2014; 2:98–107
100. Cummins EP, Taylor CT: Hypoxia-responsive transcription factors. *Pflugers Arch* 2005; 450:363–71
101. Linko R, Okkonen M, Pettilä V, Perttilä J, Parviainen I, Ruokonen E, Tenhunen J, Ala-Kokko T, Varpula T; FINNALI-Study Group: Acute respiratory failure in intensive care units. FINNALI: A prospective cohort study. *Intensive Care Med* 2009; 35:1352–61
102. Esteban A, Anzueto A, Frutos F, Alía I, Brochard L, Stewart TE, Benito S, Epstein SK, Apezteguía C, Nightingale P, Arroliga AC, Tobin MJ; Mechanical Ventilation International Study Group: Characteristics and outcomes in adult patients receiving mechanical ventilation: A 28-day international study. *JAMA* 2002; 287:345–55
103. Choi WI, Shehu E, Lim SY, Koh SO, Jeon K, Na S, Lim CM, Lee YJ, Kim SC, Kim IH, Kim JH, Kim JY, Lim J, Rhee CK, Park S, Kim HC, Lee JH, Lee JH, Park J, Koh Y, Suh GY; Korean Study Group on Respiratory Failure (KOSREF): Markers of poor outcome in patients with acute hypoxemic respiratory failure. *J Crit Care* 2014; 29:797–802
104. Lühr OR, Antonsen K, Karlsson M, Aardal A, Frostell CG, Bonde JAN: Incidence and mortality after acute respiratory failure and acute respiratory distress syndrome in Sweden, Denmark, and Iceland. *Am J Respir Crit Care Med* 1999; 159:1849–61
105. Villar J, Pérez-Méndez L, Kacmarek RM: Current definitions of acute lung injury and the acute respiratory distress syndrome do not reflect their true severity and outcome. *Intensive Care Med* 1999; 25:930–5
106. Doyle RL, Szaflarski N, Modin GW, Wiener-kronish JP, Matthay MA: Identification of patients with acute lung injury predictors of mortality. *Am J Respir Crit Care Med* 1995; 152:1818–24
107. Khandelwal N, Hough CL, Bansal A, Veenstra DL, Treggiari MM: Long-term survival in patients with severe acute respiratory distress syndrome and rescue therapies for refractory hypoxemia\*. *Crit Care Med* 2014; 42:1610–8
108. Monchi M, Bellenfant F, Cariou A, Joly LM, Thebert D, Laurent I, Dhainaut JF, Brunet F: Early predictive factors of survival in the acute respiratory distress syndrome. A multivariate analysis. *Am J Respir Crit Care Med* 1998; 158:1076–81
109. Zilberberg MD, Epstein SK: Acute lung injury in the medical ICU: Comorbid conditions, age, etiology, and hospital outcome. *Am J Respir Crit Care Med* 1998; 157(4 pt 1):1159–64
110. de Jonge E, Peelen L, Keijzers PJ, Joore H, de Lange D, van der Voort PHJ, Bosman RJ, de Waal RA, Wesselink R, de Keizer NF: Association between administered oxygen, arterial partial oxygen pressure and mortality in mechanically ventilated intensive care unit patients. *Crit. Care* 2008; 12:R156
111. Eastwood G, Bellomo R, Bailey M, Taori G, Pilcher D, Young P, Beasley R: Arterial oxygen tension and mortality in mechanically ventilated patients. *Intensive Care Med* 2012; 38:91–8
112. Network ARDS: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. Roy G Brower Michael Matthay 2000; 342:1301–8
113. Mikkelsen ME, Anderson B, Christie JD, Hopkins RO, Lanken PN: Can we optimize long-term outcomes in acute respiratory distress syndrome by targeting normoxemia? *Ann. Am. Thorac. Soc* 2014; 11:613–8
114. Hafner S, Radermacher P, Asfar P, Vincent J-L: Hyperoxia in intensive care and emergency medicine: Dr Jekyll or Mr. Hyde? An update, Annual Update in Intensive Care and Emergency Medicine 2015. Switzerland, Springer International Publishing, 2015, pp 167–78
115. Stub D, Smith K, Bernard S, Nehme Z, Stephenson M, Bray JE, Cameron P, Barger B, Ellims AH, Taylor AJ, Meredith IT, Kaye DM; AVOID Investigators: Air versus oxygen in ST-segment-elevation myocardial infarction. *Circulation* 2015; 131:2143–50
116. Suzuki S, Eastwood GM, Glassford NJ, Peck L, Young H, Garcia-Alvarez M, Schneider AG, Bellomo R: Conservative oxygen therapy in mechanically ventilated patients: A pilot before-and-after trial. *Crit Care Med* 2014; 42:1414–22
117. Helmerhorst HJF, Schultz MJ, van der Voort PHJ, Bosman RJ, Juffermans NP, de Wilde RBP, van den Akker-van Marle ME, van Bodegom-Vos L, de Vries M, Eslami S, de Keizer NF, Abu-Hanna A, van Westerloo DJ, de Jonge E: Effectiveness and clinical outcomes of a two-step implementation of conservative oxygenation targets in critically ill patients. *Crit Care Med* 2016; 44:554–63
118. Panwar R, Hardie M, Bellomo R, Barrot L, Eastwood GM, Young PJ, Capellier G, Harrigan PW, Bailey M; CLOSE Study Investigators; ANZICS Clinical Trials Group: Conservative versus liberal oxygenation targets for mechanically ventilated patients. A pilot multicenter randomized controlled trial. *Am J Respir Crit Care Med* 2016; 193:43–51
119. Cohen J, Vincent JL, Adhikari NK, Machado FR, Angus DC, Calandra T, Jaton K, Giulieri S, Delaloye J, Opal S, Tracey K, van der Poll T, Pelfrene E: Sepsis: A roadmap for future research. *Lancet Infect Dis* 2015; 15:581–614
120. Angus DC, van der Poll T: Severe sepsis and septic shock. *N Engl J Med* 2013; 369:840–51
121. Martin DS, Grocott MP: Oxygen therapy in critical illness: Precise control of arterial oxygenation and permissive hypoxemia. *Crit Care Med* 2013; 41:423–32
122. Hopkins RO, Weaver LK, Pope D, Orme JF, Bigler ED, Larson-LOHR V: Neuropsychological sequelae and impaired health status in survivors of severe acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1999; 160:50–6
123. Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP: Pharmacologic stabilization of HIF-1 $\alpha$  increases hematopoietic stem cell quiescence *in vivo* and accelerates blood recovery after severe irradiation. *Blood* 2013; 121:759–69
124. Bernhardt WM, Gottmann U, Doyon F, Buchholz B, Campan V, Schödel J, Reisenbuechler A, Klaus S, Arend M, Flippin L, Willam C, Wiesener MS, Yard B, Warnecke C, Eckardt KU: Donor treatment with a PHD-inhibitor activating HIFs prevents graft injury and prolongs survival in an allogeneic kidney transplant model. *Proc Natl Acad Sci USA* 2009; 106:21276–81
125. Bernhardt WM, Wiesener MS, Scigalla P, Chou J, Schmieder RE, Günzler V, Eckardt KU: Inhibition of prolyl hydroxylases increases erythropoietin production in ESRD. *J Am Soc Nephrol* 2010; 21:2151–6
126. Macdougall IC: New anemia therapies: Translating novel strategies from bench to bedside. *Am J Kidney Dis* 2012; 59:444–51
127. Hotchkiss RS, Monneret G, Payen D: Immunosuppression in sepsis: A novel understanding of the disorder and a new therapeutic approach. *Lancet Infect Dis* 2013; 13:260–8