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Hypoxia signaling during intestinal ischemia and inflammation

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Abstract

Purpose of review—During critical illness, alterations of intestinal blood supply and inflammatory activation can result in severe intestinal hypoxia (limited oxygen availability). Conditions of hypoxia lead to the activation of a transcriptional program that is under the control of the transcription factor hypoxia-inducible factor (HIF). In many instances, HIF-dependent alterations of gene expression represent endogenous adaptive responses that dampen pathologic inflammation and could be targeted to treat intestinal injury.

Recent findings—Post-translational stabilization of the HIF transcription factor and corresponding changes in gene expression are central to the resolution of intestinal injury. Examples for such responses that we discuss in this review include hypoxia-elicited increases in extracellular adenosine production and signaling, particularly through the A2B adenosine receptor, and intestinal protection provided by hypoxia-inducible netrin-1.

Summary—The present review focuses on HIF-elicited anti-inflammatory pathways that result in intestinal protection during critical illness. Many of these pathways represent novel therapeutic targets for attenuating multiorgan failure and critical illness. Whereas these therapeutic approaches are currently being investigated in cell culture models or in genetic mouse models, we are optimistic that at least some of these novel targets can be translated from bench to bedside in the near future.

Keywords

A2BAR; adenosine receptor; HIF; hypoxia-inducible factor; netrin-1; PHD; prolyl hydroxylase

INTRODUCTION

Intestinal injury significantly contributes to critical illness, sepsis and multiorgan failure [1▪, 2–9]. For example, recent studies from the laboratory of Dr H. Thomas Lee, Columbia University, provide strong evidence for a functional role of intestinal activation in the pathogenesis of multiorgan failure. During ischemia and reperfusion injury of the liver or the kidneys, activation of intestinal inflammatory responses sets a spiral in motion that drives multi-organ failure. Indeed, ischemia and reperfusion to peripheral organs (such as the liver) results in activation of intestinal Paneth cells, and subsequent release of cytokines

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Conflicts of interest
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such as IL-17, causing multi-organ failure [10▪▪]. These exciting studies highlight the central role of intestinal injury in the pathogenesis of sepsis and multiorgan failure. As such, therapeutic strategies that would dampen intestinal inflammation and contribute to the maintenance of the gastrointestinal barrier function and its vascular support system would be central to the treatment of sepsis, and multiorgan failure such as acute kidney injury or acute lung injury [11–14].

During intestinal injury, several mechanisms result in hypoxia of the intestinal mucosa. This leads to the activation of a hypoxia-elicited transcriptional program that is critical for intestinal adaptation to conditions of limited oxygen availability by promoting anti-inflammatory responses and the resolution of mucosal injury. It is important to point out that hypoxia and inflammation share an interdependent relationship [15▪▪,16,17]. On one hand, conditions of hypoxia are typically associated with an inflammatory phenotype, characterized by inflammatory cell accumulation and leakage across epithelial and vascular barriers [8,18▪,19▪,20–28]. Simultaneously, inflammation itself frequently triggers profound tissue hypoxia [15▪▪]. This is due to an imbalance in oxygen demand and supply of inflammatory lesions. Inflamed tissues are characterized by significant increases in their oxygen consumption, both by inflamed resident cells such as epithelia or vascular endothelia, and also by recruited inflammatory cells, such as neutrophils or macrophages [1▪,29–33]. Simultaneously, supply with metabolites such as nutrients and oxygen is decreased due to vascular occlusion or thrombosis [5,34–38]. Together, these alterations in metabolic supply and demand result in inflammation-associated tissue hypoxia. Particularly mucosal organs such as the intestine are particularly prone to develop hypoxia-induced inflammation [39,40]. This is due to their large surface area in conjunction with their complex vascular supply system [3,41,42]. Indeed, experimental studies from the laboratory of Sean Colgan, University of Colorado, were among the first studies to demonstrate that intestinal inflammation is associated with profound tissue hypoxia. For the purpose of these studies, Dr Colgan's research team utilized nitro-imidazole compounds that accumulate in hypoxic tissues and can be stained with antibodies [43]. Interestingly, these studies revealed that already at baseline conditions the intestinal mucosa stains with nitro-imidazole compounds, indicating some degree of hypoxia. Dr Colgan and his team refer to this observation as 'physiologic hypoxia of the mucosal epithelium' [15▪▪]. Indeed, this finding is not too surprising considering the fact that the intestinal lumen is anaerobic leading to an extremely steep oxygen gradient across the epithelial monolayer of the intestinal epithelium. However, nitro-imidazole staining increases profoundly in animals with experimentally induced intestinal inflammation, demonstrating that inflamed tissues become profoundly hypoxic [44].

The interdependent relationship between hypoxia and inflammation plays an important role in many diseases. Examples for disease mechanisms characterized by hypoxia-induced inflammation or by inflammation causing tissue hypoxia are summarized in Fig. 1.

TRANSCRIPTIONAL RESPONSES TO HYPOXIA

Hypoxia as occurs during intestinal ischemia or inflammation results in the activation of a transcriptional program. This transcriptional pathway involves a key transcription factor – hypoxia-inducible factor (HIF) – which is degraded under nor-moxic conditions, and is stabilized when oxygen availability is limited [45,46]. Molecular studies demonstrated that HIF is a hetero-dimer consisting of two subunits, HIF-1α and HIF-1β. Whereas HIF-1β is stably expressed, HIF-1α is highly regulated on a post-translational level. During conditions of normal oxygen concentrations, this regulatory pathway results in immediate destruction of HIF-1α via the proteasomal pathway. In fact, hydroxylation of prolyl residues by prolyl hydroxylases (PHDs) results in degradation of HIF-1α by binding of the von Hippel Lindau

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gene product (pVHL) followed by ubiquitination and proteasomal destruction [47]. In addition, factor inhibiting HIF (FIH)-dependent hydroxylation of asparagyl residues prevents co-activator binding (e.g. p300) to HIF-1α, as an additional inhibitory pathway for HIF during nor-moxic conditions [48,49]. In contrast, PHDs and FIH are inactive during hypoxic conditions, allowing stabilization of the HIF hetero-dimer, and binding of HIF to the promoter region of hypoxia-regulated genes. In fact, HIF binds to a specific consensus sequence within the promoter region of hypoxia-driven genes [so called hypoxia-responsive element (HRE)], resulting in induction or repression of gene transcription [50]. Most frequently, HIF binding results in significant induction of gene transcription, for example in the case of erythropoietin, or vascular endothelial growth factor [51]. Whereas the molecular mechanism that determines if HIF functions as inducer or repressor of gene expression remains unclear, several examples have provided evidence that HIF can also mediate gene repression under hypoxia conditions, for example in the case of the peroxisome proliferator-activated receptor α [52], the adenosine kinase [53] or the equilibrative nucleoside transporter ENT1 [21] or ENT2 [54]. HIF-dependent gene regulation involves a large array of genes important in adaptation to hypoxia, including genes central for anaerobic metabolism (e.g. glycolytic enzymes) [51,55], mitochondrial functions [56], angiogenesis (e.g. vascular endothelial growth factor) [51,57], attenuation of inflammation (e.g. the anti-inflammatory guidance molecule netrin-1) [40] or erythropoiesis (e.g. erythropoietin) [58].

It is important to point out that oxygen sensing does not occur through HIFs, but through a group of oxygen-dependent enzymes that regulate the stability of the α subunit of HIF – the so called PHDs. Under well oxygenated conditions, HIFα becomes hydroxylated at one (or both) of two highly conserved prolyl residues by members of the PHD domain family (also called EgIN family) [46,59]. Hydroxylation of either of these prolyl residues generates a binding site for the pVHL tumor suppressor protein, which is a component of a ubiquitin ligase complex. As a result, HIFα is polyubiquitylated and subjected to proteasomal degradation when oxygen is available. The PHD proteins belong to the Fe(II) and 2-oxoglutarate-dependent oxygenase superfamily, whose activity is absolutely dependent on oxygen. Accordingly, the rate of HIF hydroxylation is suppressed by hypoxia. Under low oxygen conditions, or in cells lacking functional pVHL, HIFα accumulates, dimerizes with an HIFβ family member, translocates to the nucleus, and transcriptionally activates different genes, including genes involved in erythropoiesis, angiogenesis, autophagy, and energy metabolism [45–47,49,60,61]. It is important to point out that pharmacologic inhibitors of PHDs result in the nor-moxic stabilization of HIF. Indeed, such compounds have been examined in patients; for example, a recent study in patients with renal anemia demonstrated that PHD inhibitors that function to activate HIF can be used to reverse anemia. As such, the use of pharmacologic HIF activators for the treatment of critical illness is imminent.

HYPOXIA-INDUCIBLE FACTOR-DEPENDENT GENE PRODUCTS IN INTESTINAL ISCHEMIA AND INFLAMMATION

Hypoxia-inducible factor stabilization during gut ischemia or inflammation increases adenosine signaling pathways, thereby promoting the resolution of intestinal injury and inflammation.

Extracellular adenosine generation and signaling

An important example for a hypoxia-elicited gut protection comes from the signaling molecule adenosine. In the extracellular compartment, adenosine mainly stems from precursor molecules such as ATP or ADP [2,22,26,31]. Whereas ATP can function as a signaling molecule itself and has been implicated as inflammatory mediator and danger signal [62,63], its enzymatic conversion to adenosine, and adenosine signaling events have

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been implicated in the resolution of intestinal injury and inflammation. For example, ATP conversion to adenosine occurs in a two-step process. The first step is the conversion of ATP or ADP to AMP. This process is under the enzymatic control of the ectoapyrase CD39, a hypoxia-induced enzyme that is expressed on the extracellular surface of multiple cells [64]. Mice with targeted gene deletion of *cd39* experience a more severe phenotype during intestinal inflammation [8,65] or intestinal ischemia [66]. Similarly, *cd39*$^{-/-}$ mice experience more severe organ injury in other models of ischemia and inflammation, including the kidneys [67], the heart [32,68], the liver [64], the lungs [12,69], the vasculature [8,22,24,25] or the brain [70]. Together, these studies highlight that extracellular conversion ATP/ADP breakdown is hypoxia-stimulated and serves as a control mechanism to promote intestinal injury resolution (Fig. 2).

The second step of extracellular adenosine generation signaling involves the enzymatic conversion of AMP to adenosine which is catalyzed by the 5′-ectonucleodidase CD73 – a GPI-anchored enzyme that is expressed on most cell types on their extracellular surface [11,12,28,29,71–76]. Similar to CD39, previous studies had shown that CD73 is induced by hypoxia, and that hypoxia-dependent transcriptional increases of CD73 transcript, protein and function are under the control of HIF [8]. Recent studies also addressed the role of CD73 in intestinal ischemia and reperfusion injury [75]. Interestingly, pharmacological inhibition or targeted gene deletion of CD73 significantly enhanced not only local intestinal injury, but also secondary organ injury, following intestinal ischemia and reperfusion, as measured by intestinal and lung myeloperoxidase, aspartate and alanine aminotransferase, IL-1, IL-6, and histological injury. These studies also revealed that adenosine tissue levels were increased with intestinal ischemia and reperfusion injury. In contrast, *cd73*$^{-/-}$ mice had lower adenosine levels at baseline and no increase with ischemia–reperfusion injury. Again, other studies indicate that *cd73*$^{-/-}$ mice are prone to intestinal inflammation [77]. Together, these studies highlight that CD73-dependent adenosine production is under the control of hypoxia-signaling and serves as an endogenous anti-inflammatory pathway to dampen intestinal inflammation and injury.

Other studies implicate adenosine receptor signaling in gut protection from ischemia and inflammation. Extracellular adenosine can signal through four distinct adenosine receptors, the A1, A2A, A2B or the A3AR. Profiling studies of mucosal scrapings following murine ischemia and reperfusion demonstrated selective induction of the A2BAR transcript [78]. Moreover, gene-targeted mice for the *A2BAR* showed more profound intestinal ischemia–reperfusion injury compared with controls. In contrast, *A2AAR*$^{-/-}$ mice exhibited no differences in intestinal injury compared with littermate controls. In addition, selective inhibition of the A2BAR resulted in enhanced intestinal inflammation and injury during ischemia–reperfusion. Furthermore, A2BAR agonist treatment (BAY 60–6583) [13,20,27,78–82] protected from intestinal injury, inflammation, and permeability dysfunction in wild-type mice, whereas the therapeutic effects of BAY 60–6583 were abolished following targeted A2BAR gene deletion. Taken together, these studies demonstrate the A2BAR as a novel therapeutic target for protection during gastrointestinal ischemia and reperfusion. Other studies demonstrate that the A2BAR is induced by hypoxia, and this pathway is under the control of HIF [6,74,81]. Based on previous studies indicating an anti-inflammatory role for HIF-1-elicited enhancement of extracellular adenosine production via CD73 and signaling through the A2BAR, a recent study targeted HIF-1 during intestinal ischemia and reperfusion using pharmacological or genetic approaches [74]. Initial studies with pharmacological HIF activation indicated attenuation of intestinal injury with dimethyloxallyl glycine (DMOG – a well characterized HIF activator) treatment. Moreover, DMOG treatment was associated with induction of CD73 transcript and protein, DMOG protection was abolished in *cd73*$^{-/-}$ mice. Similarly, DMOG treatment enhanced A2BAR transcript and protein levels, whereas DMOG protection was abolished in

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A2BAR$^{-/-}$ mice. Finally, studies of mice with conditional HIF-1α deletion in intestinal epithelia or pharmacological inhibition of HIF-1 with 17-(dimethylaminoethylamino)-17-deme-thoxygeldanamycin revealed enhanced tissue injury during ischemia–reperfusion. These studies indicated a tissue-protective role of HIF-dependent enhancement of intestinal adenosine generation and signaling during intestinal ischemia–reperfusion. Moreover, these findings are also consistent with studies indicating a tissue-protective and anti-inflammatory role of HIF signaling in models of intestinal inflammation [17,44,83–85,86▪▪].

In addition to enhancing extracellular adenosine production and signaling, HIF has also been implicated in enhancing the extracellular signaling effects of adenosine by transcriptionally repressing its uptake mechanisms and its metabolism via repression of equilibrative nucleoside transporters [21,38,54,87,88] and the adenosine kinase – an intracellular enzyme responsible for adenosine conversion to AMP [53]. Taken together, these studies highlight that conditions of intestinal hypoxia –such as those which occur during gut ischemia or inflammation – are associated with the stabilization of the HIF transcription factor. In turn, HIF stabilization drives a pathway that increases the extra-cellular production and signaling effects of adenosine, thereby promoting the resolution of intestinal injury and inflammation.

Neuronal guidance molecule netrin-1 in intestinal injury

Netrin-1 is a neuronal guidance molecule that is known for its functions in brain development [89]. However, previous studies had linked netrin-1 to the coordination of inflammatory responses [39,90]. Given that mucosal surfaces are particularly prone to hypoxia-elicited inflammation, recent studies sought to determine the function of netrin-1 in hypoxia-induced inflammation of the intestine [40]. These studies revealed HIF-1-dependent induction of expression of the gene encoding netrin-1 (Ntn1) in hypoxic epithelia. Neutrophil transepithelial migration studies showed that by engaging A2B adenosine receptor A2BAR on neutrophils, netrin-1 attenuated neutrophil transmigration. Exogenous netrin-1 suppressed hypoxia-elicited inflammation of the intestine in wild-type but not in A2BAR-deficient mice, and inflammatory hypoxia was enhanced in *Ntn1*$^{+/-}$ mice relative to that in *Ntn1*$^{+/+}$ mice. Together these studies demonstrate that HIF-1-dependent induction of netrin-1 attenuates hypoxia-elicited inflammation at mucosal surfaces [40]. Other studies also provide evidence for a role of netrin-1 signaling in attenuating intestinal inflammation [39]. Interestingly, both studies indicate that this protection involves signaling events through the A2BAR; at present it is not clear how netrin-1 can function through the A2BAR. Whereas direct binding of netrin-1 to the adenosine binding site of the A2BAR and concomitant signaling appears more unlikely, ongoing studies indicate that this could potentially involve an allosteric enhancement of A2BAR signaling effects.

CONCLUSION

Experimental studies utilizing murine models of intestinal ischemia and inflammation in combination with cell culture studies provide strong evidence that intestinal injury is associated with profound hypoxia of the apical aspects of the mucosa [1▪,3,5,15▪▪,17,18▪, 19▪,31,35–37]. Intestinal hypoxia is associated with the post-translational stabilization of the transcription factor HIF. Indeed, multiple studies demonstrate a protective role of HIF stabilization in models of intestinal inflammation or injury [1▪,4–9,17,44,54,74,83–85,86▪▪]. The HIF-elicited protection involves the transcriptional induction of many gene products [10▪▪]. Recent studies have identified extracellular adenosine production and signaling as a pathway that is under the direct control of HIF, and provides potent protection from intestinal injury [8,11,12,22,24,25,28,29, 32,64,67–69,71–76,91–94]. Similarly, signaling events through the neuronal guidance molecule netrin-1 have been shown to be transcriptionally induced by HIF, and are associated with mucosal protection from hypoxia-

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induced inflammation and pathologic intestinal inflammation. These experimental studies highlight that therapeutic strategies including HIF activators, pharmacologic means of enhancing extracellular adenosine production or signaling (particularly through the A2BAR) could be considered for the treatment of patients suffering from critical illness in order to promote the resolution of gut injury and to dampen pathologic intestinal inflammation [3,6,13,20,24,25, 27,30,35,37,39,71,74,78–82,92,94–96]. Presently, these therapeutic inventions have been examined in cell culture models, or in murine models of intestinal injury. We are hopeful, that at least some of these therapeutic targets can be translated from bench to bedside, and will eventually be introduced into the treatment of patients suffering from critical illness.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 218).

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KEY POINTS

- Intestinal injury plays a key role in the pathogenesis of sepsis and multiorgan failure. Recent studies indicate that this could involve activation of intestinal Paneth cells and subsequent cytokine release.
- During intestinal injury (ischemia, inflammation), the intestinal mucosa becomes profoundly hypoxic resulting in the post-translational stabilization of the hypoxia-adaptive transcription factor HIF.
- HIF stabilization is associated with a transcriptional program that enhances hypoxia tolerance, attenuates intestinal inflammation and promotes the resolution of injury.
- Pharmacologic strategies to achieve HIF stabilization (e.g. PHD inhibitor) are protective during intestinal inflammation or ischemia and reperfusion.
- HIF-target genes that have been implicated in intestinal protection include the extracellular production of adenosine and its signaling events through the A2BAR. These resemble novel pharmacologic targets for the prevention or treatment of intestinal injury during critical illness.
- The evidence for these findings discussed in this review comes mostly from studies in tissue culture models, or murine models of intestinal injury. It will be critical to translate these experimental findings from bench to bedside for the treatment of critical illness.

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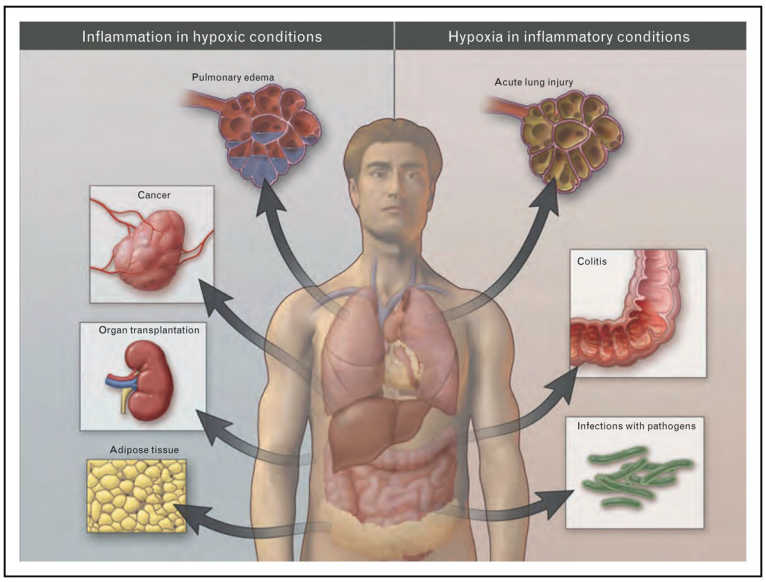

FIGURE 1.

Overview of clinical conditions characterized primarily by tissue hypoxia resulting in inflammatory changes (left), or inflammatory diseases that lead to tissue hypoxia (right; from the New England Journal of Medicine with permission [19▪]).

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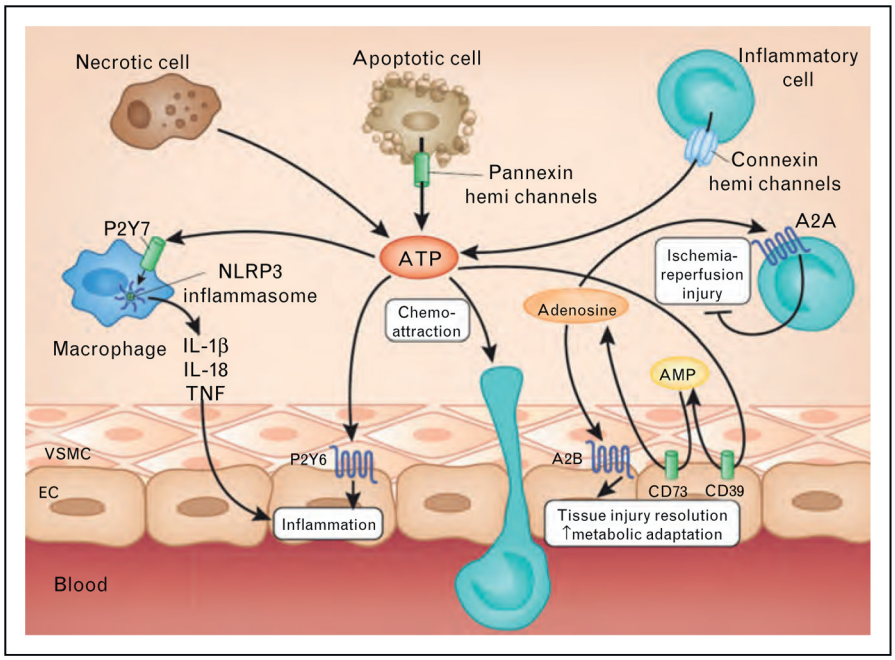

FIGURE 2.

Extracellular signaling of ATP and adenosine during ischemia or inflammation. Multiple cell types release ATP during ischemia and reperfusion (e.g. spillover from necrotic cells or controlled release through pannexin hemichannels from apoptotic cells or connexin hemichannels from activated inflammatory cells). Subsequent binding of ATP to P2 receptors enhances pathological inflammation and tissue injury, for example, through P2X7-dependent Nlrp3 inflammasome activation and P2Y6-dependent enhancement of vascular inflammation. ATP can be rapidly converted to adenosine through the ecto-apyrase CD39 (conversion of ATP to AMP) and subsequently by the ecto-5′ nucleotidase CD73 (conversion of AMP to adenosine). Adenosine signaling dampens sterile inflammation, enhances metabolic adaptation to limited oxygen availability and promotes the resolution of injury through activation of A2A adenosine receptors expressed on inflammatory cells and activation of A2B adenosine receptors expressed on tissue-resident cells (e.g. cardiac myocytes, vascular endothelia or intestinal epithelia). EC, endothelial cell; VSMC, vascular smooth muscle cell. (from Nature Medicine with permission [18▪]).