

Oxygen metabolism and barrier regulation in the intestinal mucosa

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Mucosal surfaces are lined by epithelial cells and provide an important barrier to the flux of antigens from the outside. This barrier is provided at a number of levels, including epithelial junctional complexes, mucus production, and mucosa-derived antimicrobials. Tissue metabolism is central to the maintenance of homeostasis in the mucosa. In the intestine, for example, baseline pO₂ levels are uniquely low due to counter-current blood flow and the presence of large numbers of bacteria. As such, hypoxia and HIF signaling predominates normal intestinal metabolism and barrier regulation during both homeostasis and active inflammation. Contributing factors that elicit important adaptive responses within the mucosa include the transcriptional regulation of tight junction proteins, metabolic regulation of barrier components, and changes in autophagic flux. Here, we review recent literature around the topic of hypoxia and barrier function in health and during disease.

Introduction

The mucosa provides a physical barrier between biologic compartments, preventing the free mixing of luminal antigenic material with the lamina propria, which houses the mucosal immune system. The establishment and maintenance of a selectively permeable barrier occurs through interactions of the extracellular domains of multiple transmembrane adhesion proteins between adjacent cells (adherens junction, tight junction, gap junction) or between the mucosal layer and extracellular matrix components (1). These interactions not only determine the physical integrity of the tissue, but also establish the physical organization of lipids and proteins within the plasma membrane in a polarized fashion.

The epithelium overlying all mucosal tissues is supported by a rich vasculature. In these settings, even small perturbations in blood flow can result in relatively large decreases in O₂ delivery (hypoxia) to the supporting epithelium. Studies in the mucosa have provided important insight into metabolic demands associated with inflammatory responses. The GI tract, for example, is characterized by a highly unique oxygenation profile, wherein the small and large intestine adapt to significant fluctuations in blood perfusion on a regular basis (2). Even at baseline, epithelial cells lining the mucosa exist at a relatively low pO₂, herein described as “physiologic hypoxia” (3). Elucidation of countercurrent O₂ exchange mechanisms in the small intestine has revealed that O₂ from the arterial blood supply diffuses to adjacent venules along the crypt villus axis, resulting in graded regions of significant hypoxia (4). In the colon, a steep O₂ gradient exists from the anaerobic lumen, across the epithelium, and into the vascularized subepithelial mucosa (5). Given the high-energy requirement of the gut and the integral role of the epithelium in maintaining intesti-

nal homeostasis, it is not surprising that these tissues have evolved a number of mechanisms to cope with this austere metabolic environment. Here, we discuss how the intestinal mucosa functionally adapts to the low pO₂ environment in health and during disease.

Oxygen utilization in the mucosa

A comparison of mucosal tissue oxygenation reveals some rather stark contrasts. Fine measurements of the healthy lung alveolus have revealed a surface pO₂ of 100 to 110 mmHg (6). By contrast, the most luminal aspect of the healthy colon exists at a pO₂ of less than 10 mmHg (5, 7). Such differences reflect a combination of O₂ sources, the anatomy of blood flow within the organ, and the presence of commensal microbes (8).

Local pO₂ environments can be tracked in vivo using 2-nitroimidazole dyes, a class of compounds that undergo intracellular metabolism dependent on levels of cellular O₂ content (9, 10). Nitroimidazoles form adducts with thiol groups in proteins, peptides, and amino acids. These adducts are retained at pO₂ levels of less than 10 mmHg and have the added advantage of imaging only viable tissue (11). Localization of nitroimidazole adducts using antibodies has resulted in two observations (see Figure 1). First, in the normal GI mucosa, particularly in the colon, physiologic hypoxia predominates (7). These studies have shown that these low pO₂ conditions are critical for the constitutive expression of some innate immune factors (12) and certain epithelial tight junction proteins (13) (discussed below). Second, inflammatory lesions observed in models of colitis are profoundly hypoxic (or even anoxic), with pO₂ levels that are comparable to the pO₂ levels observed in some large tumors (7). While there are multiple contributing factors (i.e., increased O₂ consumption, vasoconstriction, edema) that result in decreased O₂ delivery and resultant hypoxia (7), it was recently shown that a major component of deep tissue hypoxia in active inflammation is derived from NADPH oxidase-dependent O₂ consumption by activated leukocytes, particularly neutrophils (14).

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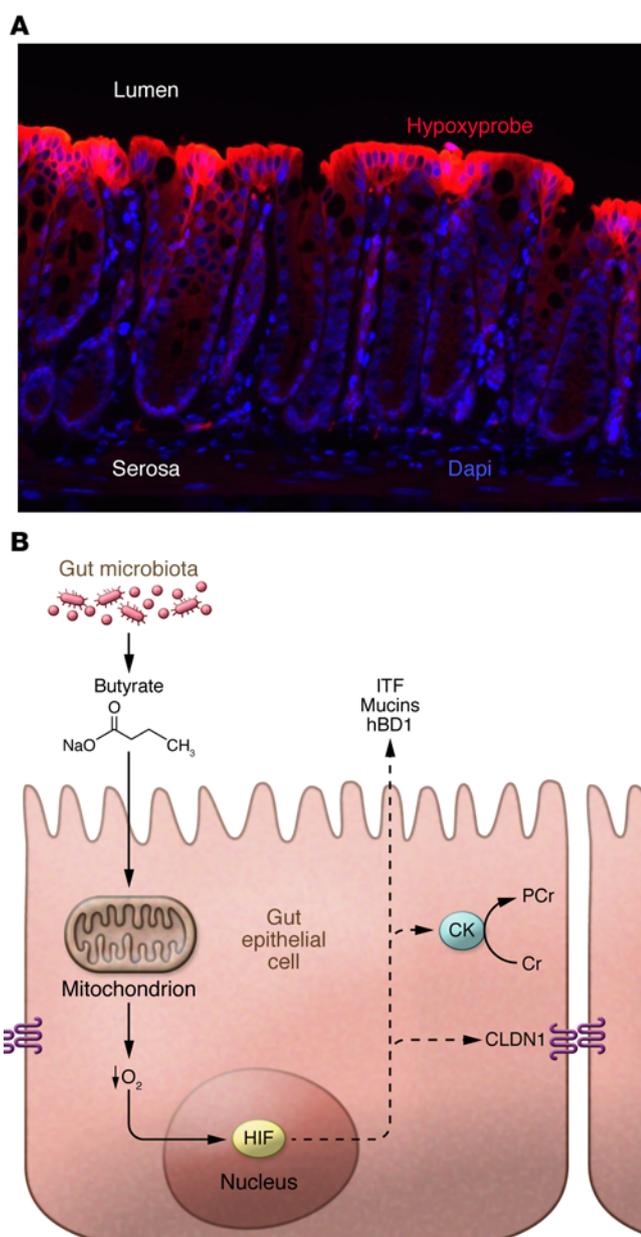


Figure 1. Physiologic hypoxia and barrier regulation in the healthy intestinal mucosa. (A) Localization of low pO_2 along the crypt-villus axis and the presence of “physiologic hypoxia.” Colonic mucosa of healthy mice retain small amounts of nitroimidazole adduct (Hypoxyprobe) that is detected along the luminal aspect of the colon (red). Blue depicts nuclei counterstaining with Dapi. (B) A mechanism in which microbially derived SCFAs, such as butyrate, elicit increased O_2 consumption to the extent that HIF is stabilized and transcriptionally active. Also shown here are barrier-related pathways that are directly regulated by HIF. (Cr, creatine; PCr, phosphocreatine; HIF, hypoxia-inducible factor; CK, creatine kinase; CLDN1, claudin-1; ITF, intestinal trefoil factor).

ligase proteasomal targeting (18). To date, three HIF- α subunits have been identified, with the highest level of sequence conservation between HIF-1 α and HIF-2 α (19). Despite their concurrent expression in many cell types, HIF-1 and HIF-2 play nonredundant roles (20) that appear to be highly cell specific to facilitate both short- and long-term adaptations to hypoxia (21). A second HIF activity controller operates in the carboxy terminal transactivation domain of HIF- α subunit, where hypoxia blocks the hydroxylation of Asp80, thereby facilitating the recruitment of CBP/p300 (22).

Molecular aspects of barrier regulation in the mucosa

As previously mentioned, a major function of the mucosa is the provision of a physical barrier between the inside and outside world. This barrier is maintained by cell-cell interactions known as tight junctions (TJs), which serve to seal together the epithelial monolayer (2). TJs are composed of both cytosolic (i.e., zonula occludens) and integral membrane proteins (i.e., occludin, claudins). Adjacent to the TJ is the adherens junction (AJ), composed of E-cadherin and cadherin/catenin complexes that are linked with the actin cytoskeleton (23). The cytoskeletal links that support apical epithelial junctions are among the most highly ordered arrays of actin filaments in nature (24). The circumferential actomyosin ring mediates selective barrier function in both health and disease (25) and is disrupted by diverse inflammatory stimuli (26, 27). In the GI mucosa, such a barrier exists in the presence of trillions of microbes that play a symbiotic role within the host and also serve as a source of disease during dysbiosis (discussed below). Here we discuss the contributions of various components to barrier function and their regulation by the hypoxic microenvironment that has come to characterize the mucosal interface (summarized in Figure 1).

Adherens junctions and hypoxia. Older evidence from both ischemic organs and ATP depletion models described the dissolution of AJ complexes (28), likely initiated by the hyperphosphorylation of the catenins (29). During recovery, epithelia depend on reassembly of the AJ proteins, which necessitates resynthesis of E-cadherin, reassembly of catenins, and reformation of functional AJs. Considering misfolding and/or aggregation of membrane proteins such as E-cadherin in hypoxia and/or ATP depletion, this could be a limiting factor in the face of sustained hypoxia. Conversely, it has been argued that in some tumors, microenvironmental factors such as hypoxia may result in downregulation of functional surface AJ complexes and facilitate a metastatic disease phenotype (30).

Given the substantial shifts in metabolism and O_2 availability during inflammation, a number of studies have shown that stabilization of HIF in low O_2 triggers the expression of genes that enable epithelial cells to function as an effective barrier (15). HIF is a member of the Per-ARNT-Sim (PAS) family of basic helix-loop-helix (bHLH) transcription factors and is considered one of the central regulators of tissue metabolism (16). HIF activity is dependent on modifications to the O_2 -dependent degradation (ODD) domain expressed on the α subunit and subsequent nuclear translocation to form a functional complex with HIF-1 β and cofactors such as CREB-binding protein (CBP) and its ortholog p300 (17). Under conditions of normal O_2 supply, iron and O_2 -dependent hydroxylation of two prolines (Pro564 and Pro402) within the ODD of the HIF- α subunit initiates the association with the von Hippel-Lindau tumor suppressor protein (pVHL) and rapid degradation via ubiquitin-E3

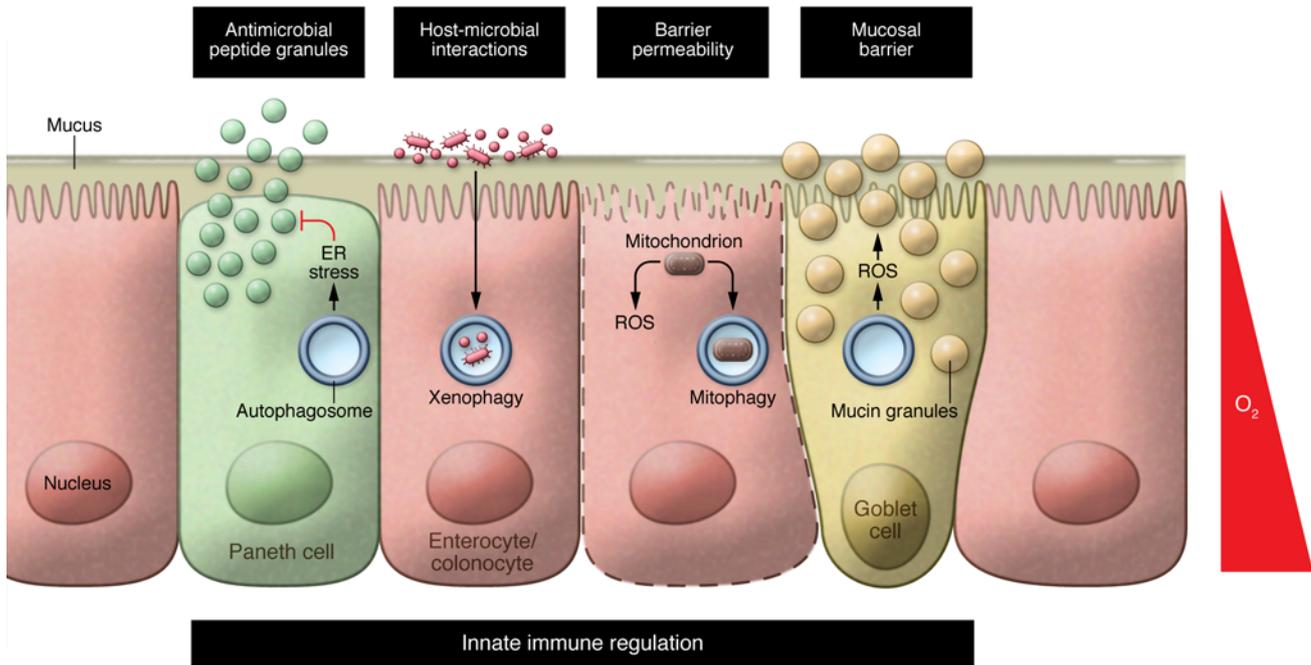


Figure 2. Epithelial autophagy and barrier function. IBD results from dysregulated microbial, epithelial, and immune cell interactions. Autophagy is a key host defense mechanism for epithelial barrier protection against invasive bacteria and metabolic stressors during mucosal inflammation. IBD-associated mutations in autophagy genes have been linked to dysregulated IEC mitophagy, xenophagy, goblet cell mucus secretion, and paneth cell ER stress responses. Although the role of HIF in IEC-intrinsic autophagy *in vivo* has not yet been characterized, HIF has been identified as a transcriptional regulator of mitochondrial autophagy and xenophagy of IBD-associated AIEC under hypoxic conditions *in vitro*.

β -Catenin signaling through T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors plays a notable role within AJs and in barrier regulation during active mucosal inflammation (31). Studies derived from microarray analysis of pulmonary epithelial cells following neutrophil (polymorphonuclear leukocyte [PMN]) transmigration revealed epithelial gene programming that results in activated β -catenin signaling and the restoration of epithelial barrier function (31). Such signaling required leukocyte elastase-dependent cleavage of E-cadherin. Parallel studies in the GI mucosa revealed that PMN transmigration elicited a prominent induction of HIF target genes within the epithelium (14). Studies utilizing HIF reporter mice, strategies that deplete circulating PMN, and transgenic mice lacking a respiratory burst (*Gp91^{phox}-/-*), revealed that recruited neutrophils rapidly deplete the local milieu of molecular O_2 in a NADPH oxidase-dependent manner. It was shown that transmigrating PMNs “transcriptionally imprint” a molecular signature that reflects PMN-mediated stabilization of HIF and transactivation of HIF targets in the surrounding tissue. This molecular signature promotes an adaptive HIF-dependent resolution of acute inflammation. Indeed, *Gp91^{phox}-/-* mice developed more severe colitic responses compared with WT mice, including exaggerated PMN infiltration, diminished inflammatory hypoxia, and increased microbial invasion. Clinical corollaries exist for this model. For example, patients that lack a functional NADPH oxidase (i.e., chronic granulomatous disease [CGD]) often present with an inflammatory bowel disease-like (IBD-like) syndrome (32). Approximately 40% of CGD patients develop this IBD-like syndrome (33), suggesting that CGD-associated IBD could represent a failure to resolve acute intestinal insults. Like-

wise, a number of SNPs that impact the NADPH oxidase complex are IBD candidate genes that are involved in very early onset IBD (34). This NADPH oxidase complex is responsible for the generation of ROS, which are used by innate immune cells (especially PMNs and monocytes/macrophages) as antimicrobial weapons. These studies provide new insight into how cells communicate within tissues and how such information might be harnessed for therapeutic purposes.

Original studies using ATP depletion models revealed an important role for high-energy phosphates in the regulation of barrier function (35, 36). These studies have prompted further analysis of hypoxic adaptation to such conditions. Notably, cytosolic creatine kinase (CK) genes are HIF-2-selective targets that are expressed within the AJs of confluent intestinal epithelia, and multiple lines of evidence indicate that CK contributes fundamentally to the energy requirements of barrier regulation (37). The CK pathway is often neglected in energy metabolism, as it is assumed that high-energy phosphate exchange between sites of ATP production (mitochondria/glycolysis) and ATP consumption (ATPases) rely primarily on the diffusion of ATP and ADP. While this may hold true in tissues devoid of CK and phosphocreatine, it is clearly inadequate in tissues with fluctuating energy demands (38). Studies in the mucosa revealed that each of the CK subunits is expressed in cultured intestinal epithelial cell lines, murine colonic epithelia, and in human colonic epithelia (37). During periods of high energy demand, the phosphocreatine pool and CK work to maintain constant concentrations of ATP and ADP. As such, CK functions as a buffer for cytosolic phosphorylation potential that appears to be critical for the proper functioning of a variety of cellular ATPases

(38). High energy expenditure within the mucosa is particularly relevant during the restoration of epithelial cells following insult (e.g., active inflammation). The dynamic regulation of epithelial junctions is tightly linked to the circumferential F-actin belt (39). From this perspective, it is not surprising that creatine/phosphocreatine may well be central to energy homeostasis and tissue barrier function during episodes of mucosal inflammation.

Tight junctions and hypoxia. Through interactions with the intracellular cytoskeleton, TJs form the backbone for the structural integrity of the epithelial barrier and constitute the physical basis for a permeability barrier to solutes and ions (40). Furthermore, TJs contribute to the polarized phenotype of epithelia by preventing lipid diffusion between apical and basolateral membrane domains, the so-called “fence function” (40). The TJ is composed of both transmembrane and peripheral membrane proteins tightly linked to the actin-based cytoskeleton (39). TJ complex assembly and transcriptional control of its components are tightly regulated by a variety of physiological and pathophysiological stimuli (1). Ischemia and hypoxia dramatically influence TJ integrity, resulting in loss of transepithelial electrical resistance, which has been observed both in ATP depletion models (41) and in vitro hypoxia models (42–44). Some of the permeability changes are attributable to alterations in the distribution of occludin, zonula occludens-1 (ZO-1), ZO-2, and cingulin (36, 41). Furthermore, TJ integrity is influenced by perturbations of the interaction with the actin-based cytoskeleton (35, 45) and by the degradation of membrane cytoskeletal proteins such as ankyrin and fodrin in ATP depletion models (46).

Claudins are a large family of tetraspanning integral membrane proteins that are uniquely responsible for the selective permeability of TJs (40). Claudins are categorized as “leaky” or “tight” with regard to their effect on barrier function (47). Claudin-1 (*CLDN1*) is an important “tight” claudin and has been shown to be dysregulated in a variety of human diseases, including IBD (40). In a recent screen of TJ targets, *CLDN1* was identified as a central mediator of aberrant junctional morphology in HIF-1 β -deficient intestinal epithelial cell (IEC) lines (13). Using both loss- and gain-of-function approaches, this work showed that HIF maintains *CLDN1* expression through binding hypoxia response element (HRE) sequences in the gene promoter. The reintroduction of *CLDN1* into HIF-1 β -deficient cells restored barrier function and morphologic abnormalities. Moreover, in vivo analysis revealed the importance of HIF-mediated *CLDN1* expression during experimental colitis. These results identify a critical link between HIF and specific TJ function, providing important insight into mechanisms of HIF-regulated epithelial homeostasis (13).

Mucus expression and hypoxia. The intestinal epithelium extends its barrier apically through formation of a mucus layer. Mucus is a complex mixture of glycoproteins secreted by goblet cells that allows delivery of nutrients to the epithelium while preventing direct exposure to luminal contents. At least 10 distinct gel-forming and surface mucins are secreted by goblet cells (48). In healthy mucosa, mucus consists of an adherent layer that is devoid of bacteria and a thicker superficial layer that is many times the diameter of the epithelium (49, 50). Hypoxia and HIF regulate several components of the mucus layer. *MUC3* is a HIF-1 α target whose product, mucin-3, colocalizes with intestinal trefoil factor (ITF), another barrier-protective molecule characterized

by robust trefoil domains (51, 52). Young and colleagues analyzed the 5'-flanking sequences of mammalian *MUC5AC* orthologs and identified evolutionarily conserved regions within domains proximal to the mRNA coding region (53). In particular, SMAD4 and HIF-1 α bind to the promoter, and mutation of their recognition motifs abolishes promoter function (53).

An under-appreciated function of the mucus layer is to provide a reservoir for secreted antimicrobial peptides, such as defensins (54). Defensins are a class of cysteine-rich antimicrobial peptides that possess broad antimicrobial activity (55, 56). Human β defensin-1 (hBD1) is notable within the intestinal epithelium because it is constitutively secreted, whereas other antimicrobial peptides are induced by inflammatory mediators (8). Constitutive expression of hBD1 depended on basal HIF-1 α signaling in multiple IEC lines and hBD1 expression correlated with other HIF target genes in human tissues (12). Another distinguishing feature of hBD1 is that the full spectrum of its antimicrobial activity is only revealed when its disulfide bonds are reduced (57). Reduction of the hBD1 disulfide bonds is accomplished by thioredoxin, which colocalizes with hBD1 in the colonic mucus; oxidation of hBD1 is prevented by the low pO₂ environment of the lumen (58). Thus, hypoxia and HIF appear to provide important regulatory roles for the expression and function of the mucus layer at multiple levels.

Tissue metabolism, hypoxia, and barrier. Mucosal barrier restitution, coupled with the dynamic regulation of the circumferential F-actin belt during barrier maintenance, requires extensive utilization of the energetically costly actin/myosin cytoskeletal structure (15, 39, 59, 60). As alluded to above, ATP and its associated creatine/phosphocreatine buffering system are metabolites of paramount importance, as exemplified by HIF transactivation of creatine transport and creatine kinase enzymes (37). Glutamine is also a fundamental component of mucosal cell metabolism and promotes barrier maintenance by transcriptional regulation of proteins involved in cell proliferation, differentiation, apoptosis, autophagy, protein turnover, antioxidant activity, and immunity (61). Other metabolites have demonstrated roles in barrier restoration and maintenance (62), including arginine and tyrosine through focal adhesion kinase-dependent signaling mechanisms (63–65), polyamines biosynthesized from ornithine (66, 67), n-3 and n-6 fatty acids (68, 69), vitamin D (70, 71), and zinc (72). In indirect support of mucosal homeostasis, cysteine, serine, proline, and especially threonine induce mucin synthesis, and thus aid barrier function by bolstering formation of this first line of defense against microbial invasion (73, 74).

Certain metabolites also participate in intestinal mucosal cell barrier maintenance by way of inflammation. Notably, HIF regulates the generation of extracellular adenosine via upregulation of CD39 and CD73 and this increase in adenosine enhances antiinflammatory signaling through binding of the HIF-inducible adenosine A2B receptor (75). Another example is the tryptophan metabolite kynurenate, which exerts antiinflammatory properties by inhibiting xanthine oxidase, resulting in decreased production of ROS during hypoxic stress (76). Likewise, glutamate, methionine, and threonine appear to improve colonic mucosal regeneration after insult (77). These examples provide insight into the integrated responses necessary for homeostatic regulation of tissue barrier function.

Host-microbial metabolism and tissue barrier

The gastrointestinal tract is home to trillions of microbes that include bacteria, fungi, and viruses. A finely regulated symbiosis exists within the mucosa. In addition to aiding in digestion, microbial communities produce a number of vitamins and fuel sources in the form of short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate. Butyrate can reach luminal concentrations of 30 mM in the colon and represents a preferred metabolic substrate for colonic epithelial cells, and up to 30% of energy may be derived from butyrate in the healthy colon (78). SCFAs are efficiently absorbed and metabolized by the epithelium (79). In contrast to other SCFAs, very little butyrate is released into portal circulation (78). Butyrate-derived acetyl-CoA is made available for oxidative phosphorylation and becomes a prominent fuel for colonic epithelial cells (80). It was recently revealed by Kelly et al. that butyrate increases epithelial O₂ consumption to the extent that cells sense metabolic hypoxia and HIF is stabilized (81). These same studies revealed that the lack of microbiota (using germ-free or antibiotic-treated mice) resulted in the depletion of butyrate and loss of HIF stabilization. The HIF response was rescued by butyrate supplementation. These observations were recently validated and extended to reveal that the depletion of butyrate-producing species depletes local oxygen levels and allows the expansion of aerobic luminal microbes (82). Given the multiple levels of protection afforded by HIF within the mucosa (2), such findings implicate this butyrate/HIF axis as a host/microbe crosstalk pathway wherein SCFAs promote protective signaling in the distal gut.

A homeostatic role for SCFAs in the distal gut during inflammation is likely important for disease (78, 83). For example, recent studies investigating dysbiosis in patients with IBD identified lower levels of colonic butyrate and reduced abundance of butyrate-producing organisms (e.g., certain *Faecalibacterium* and *Roseburia* genera) with disease (84–86). The importance of butyrate as the preferred epithelial substrate has been highlighted by the finding that mice with mitochondrial polymorphisms resulting in increased oxidative phosphorylation activity are resistant to colitis (87) and inhibition of β -oxidation elicits colitis-like symptoms (88). It is notable that administration of exogenous butyrate promotes resistance to experimental colitis (89, 90), and several trials have evaluated the efficacy of butyrate in the treatment of human, primarily ulcerative, colitis with mixed results (78). Finally, recent studies have indicated that SCFAs can signal through GPCRs to mediate their biological functions (91, 92). In mice, deletion of *Gpr41* and *Gpr43* mediates protective immunity in inflammatory models (91, 92). Treatment of mice with propionate promotes colonic protection during inflammation (92) and the major butyrate receptor GPR109a functions to suppress inflammation-associated carcinogenesis (93).

It is notable that other studies have implicated microbial regulation of iron availability as a mechanism of HIF regulation in the intestine. Pathogenic gram-negative bacteria acquire iron from the host through the secretion of siderophores, which chelate iron for incorporation into normal biological processes. Holden et al. demonstrated that bacterial siderophores such as enterobactin chelate iron to the extent that HIF is stabilized and HIF target genes are induced (94). Likewise, it was shown that siderophores from a number of bacterial genera (e.g., *Salmonella*, *Yersenia*, *Enterobacter*) are capable of stabilizing HIF (95). It was

also demonstrated that such HIF stabilization within the intestinal epithelium appears to be an adaptive defense mechanism, since mice lacking epithelial HIF-1 α were more susceptible to *Yersinia* infection. Such studies provide examples of hypoxia-independent activation of HIF pathways that likely have important biological implications in the GI mucosa.

Autophagy and the regulation of barrier function

The advent of GWAS has greatly enhanced our understanding of the genetic basis of diseases such as IBD and has highlighted the cell-intrinsic host response to enteric bacteria as a central paradigm of disease pathogenesis (96). Variants in several genes that regulate the autophagy pathway have emerged as significant risk alleles for IBD, including autophagy-related 16-like 1 (*ATG16L1*) (97, 98) and immunity-related GTPase family M (*IRGM*) (99, 100). Moreover, metabolic shifts associated with mucosal inflammation have been shown to directly influence autophagic responses, including ER stress, ROS generation, and hypoxia.

Autophagy is traditionally viewed as a primordial cellular degradation pathway that facilitates cell survival under conditions of nutrient deprivation or metabolic stress (101). Cytoplasmic targets are engulfed by a double-membrane vacuole 0.5 to 1.0 μ m in diameter termed the autophagosome, which subsequently fuses with lysosomes (autolysosome) for resident hydrolase-mediated digestion. Autophagy also mediates selective recruitment and degradation of cellular targets such as senescent or damaged organelles, protein aggregates, and invading microorganisms, whose large size precludes their elimination by the proteasome. Although a marked effort has been made in recent years to define protein complexes and signaling events underlying acute autophagosome formation, regulation of these processes at the transcriptional level is poorly understood, particularly in the intestine. A number of transcription factors are known to orchestrate altered expression of autophagy genes in response to different microenvironmental stimuli, including transcription factor EB (TFEB) (102, 103), zinc finger with KRAB and SCAN domains 3 (ZKSCAN3) (104), AMPK (105), and HIF-1 (21, 106).

Perturbations in autophagy disrupt multiple aspects of barrier function (see Figure 2). Recent work has defined a central role for autophagy in colonic goblet cell mucus secretion. Conditional epithelial knockout studies of *Atg5*, *Atg7*, and *Lc3b* in mice revealed that autophagy is required for efficient mucin granule accumulation and secretion from goblet cells (107). Interestingly, this work implicates NADPH oxidase-derived ROS at the autophagosome-endosome interface as critical mediators of mucus secretion. The NLRP6 inflammasome has also recently been linked to autophagy-dependent mucus secretion, with *Nlrp6*^{-/-} mice displaying defective goblet cell autophagy, abrogated mucus granule exocytosis, and high susceptibility to enteric infection (108). Importantly, even partial deficiency of the core autophagy protein ATG5 recapitulates goblet cell and mucus layer defects (108), while mice harboring the prevalent Crohn's disease-associated *Atg16l1* mutation T300A were found to display morphological anomalies in epithelial cuff colonic goblet cells (109).

Similarly, defects in IBD-linked autophagy gene expression have been closely connected to disrupted granule exocytosis from Paneth cells (Figure 2). This specialized ileal epithelial lineage

functions in part to regulate the intestinal microbiota through the secretion of antimicrobial peptides, such as defensins. Hypomorphic expression of *Atg16l1* (*Atg16l1^{HM}*) in mice resulted in profound Paneth cell abnormalities including disorganized and diminished granules, diffuse lysozyme staining, and altered transcriptional profiles leading to increased expression of inflammatory mediators (110). Importantly, similar Paneth cell defects have been reported for knockin transgenic mice and CD patients homozygous for the ATG16L1 T300A risk allele (109, 111). Although *Atg16l1^{HM}* mice do not develop spontaneous intestinal inflammation, recent work revealed that epithelial ATG16L1-mediated autophagy is necessary to dampen inositol-requiring enzyme 1 α -regulated (IRE1 α -regulated) NF- κ B activation and attenuate ER stress and cell death in intestinal epithelia deficient in X-box binding protein 1 (*Xbp1*) and the unfolded protein response (UPR) (112). This seminal study highlights a critical interplay between commensal microbiota, the UPR, and autophagy in Paneth cells, such that dysregulated UPR and increased ER stress may in fact define the initiating threshold for intestinal inflammation in patients harboring certain autophagy gene defects.

The role of absorptive enterocytes as innate immune sensors of bacterial invasion has now been demonstrated in a number of in vivo studies (113–115). Invasive pathogenic and commensal species that penetrate the mucus layer and evade antimicrobial peptides can enter the IEC monolayer and replicate intracellularly. As such, IEC-intrinsic mechanisms that limit bacterial replication and systemic dissemination have been the focus of intense investigation, particularly those involved in xenophagy. Altered expression of xenophagic proteins such as IRGM have been noted in epithelial cells from patients with active IBD (116), and cell culture models of the ATG16L1 variant T300A display defective xenophagy of internalized pathogens (117, 118). Conditional deletion of *Atg16l1* or *Atg5* within the IEC compartment in mice resulted in impaired epithelial autophagy with increased numbers of intracellular *Salmonella enterica* serovar Typhimurium and increased dissemination to systemic sites (113, 114), highlighting an essential role for IEC xenophagy in pathogen clearance. Enterocyte autophagy is likely a specialized response to invasive microbes, as germ-free mice infected with invasive, gram-positive commensal *Enterococcus faecalis*, but not noninvasive *Lactobacillus salivarius*, displayed bacterial targeting to autophagosomes (113). The Crohn's disease-associated opportunistic pathogen adherent-invasive *Escherichia coli* (AIEC) is also a target of xenophagy in vitro, and recent work has unveiled an interesting link between HIF-1 expression and AIEC survival in cultured IECs (119). Modulation of IEC metabolism and HIF signaling by inflammatory hypoxia and/or microbial species may therefore define a conserved innate response for host protection through autophagic bacterial capture.

Metabolic regulation of epithelial autophagy

As the mucosal barrier displays such a uniquely dynamic metabolic and oxygenation profile, an important question is how tonally and microbially induced metabolic fluctuations influence autophagy in IECs. Cellular stressors such as nutrient deprivation, ROS, hypoxia, and ER stress are indeed hallmarks of pathogen invasion, but also reflect the spectrum of microenvironmental fluctuations experienced by IECs.

Although the role of HIF in physiologic and inflammatory IEC-intrinsic autophagy in vivo remains largely uncharacterized, strong evidence exists for integrated influences of the immune repertoire, bacteria, and micronutrients on hypoxic IEC signaling. Hypoxic mucosal macrophages from IBD patients exhibit increased HIF-1 and Wnt1 expression, and coculture models revealed that macrophage-derived Wnt1 can activate mTOR and inhibit autophagy in IECs (120). Mucosa-associated microbiota are likely also prominent modulators of potential hypoxia-elicited autophagic responses (see Figure 2). Both commensal and pathogenic species have been shown to influence mucosal pO₂ and/or enterocyte HIF signaling (95, 121, 122), and ablation of epithelial HIF-1 increases susceptibility to pathogen infection in a number of studies (95, 121). Reciprocally, intestinal oxygenation directly shapes the composition of gut microbial communities, and oxidative changes in intestinal inflammation may underlie the dysbiosis characteristic of IBD (5). The recent characterization of iron recycling by autophagy (ferritinophagy) (123) has additional important implications for HIF signaling and pathogen defense at the mucosal barrier. Finally, mitochondrial autophagy is an important determinant of cell viability under hypoxic stress (124, 125). Mitochondrial dysfunction, for example, has been described in IBD case studies (126–128). As outlined above, considerable overlap exists between cellular stimuli for selective autophagy of damaged mitochondria (self) and invading microbes (non-self), and identification of the ubiquitin ligase parkin as a common mediator of mitophagy and xenophagy has led to a paradigm shift in concepts of mammalian autophagy initiation (129).

Therapeutic implications

Given the breadth of diseases that are potentially influenced by HIF activity (e.g., inflammation, ischemia, edema, fibrosis) there is significant interest in the development of pharmacologic compounds that stabilize HIF and enhance the expression of HIF target genes (130). There is, in fact, a significant unmet need for treatment of diseases where blood flow is compromised and tissues become hypoxic. In the GI mucosa, such diseases include ischemic bowel disease, necrotizing enterocolitis, and IBD. Targeting HIF in these instances holds great promise in preserving tissue function and promoting wound healing and recovery (131).

One pharmacologic approach to achieve HIF stabilization is through the inhibition of HIF prolyl hydroxylases (PHDs) (132, 133). Targeting the catalytic domain of PHDs was originally achieved by screening for molecules that interfere with activation cofactors such as 2-oxoglutarate (e.g., dimethylxalylglycine [DMOG]) (134). The original studies using PHD inhibition in GI inflammation identified a protective role for pharmacologic HIF stabilization in different models of intestinal inflammation. The use of DMOG for the treatment of colitis proved highly effective in abrogating mucosal inflammation (135). A parallel study revealed that a PHD active site blocker (FG-4497) (134) was associated with the abrogation of mucosal inflammation, most prominently related to enhanced barrier function (refs. 135, 136, and see Figure 1). Additional HIF-1-selective stabilizers, such as AKB-4924, have shown antimicrobial actions through enhanced innate immunity

and likewise demonstrate significant protection in colitis models (14, 137, 138). Results from these studies in preclinical models indicate that GI diseases may be one of the more promising applications for PHD inhibitor-based therapies.

Conclusions

The stark differences in baseline O₂ tension between mucosal tissues and the profound increase in energy requirements within inflammatory lesions provide a unique opportunity to understand tissue metabolism in health and disease. Studies in model disease systems have provided new insights leading toward a better understanding of inflammatory responses and mechanisms that promote resolution. Of particular relevance is the shift in tissue oxygenation toward hypoxia, and specifically HIF-target pathways that are strongly associated with tissue barrier function and

metabolism that contribute fundamentally to inflammatory resolution. A more precise understanding of the common molecular cues, transcriptional programs, and metabolic milieu that selectively regulate the mucosal barrier will provide substantial insight into the role of IECs as innate immune sensors.

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