HHS Public Access

Author manuscript

Nat Rev Gastroenterol Hepatol. Author manuscript; available in PMC 2021 March 01.

Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2019 February ; 16(2): 106–120. doi:10.1038/s41575-018-0079-5.

Control and dysregulation of redox signalling in the gastrointestinal tract

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Abstract

Redox signalling in the gastrointestinal mucosa is held in an intricate balance. Potent microbicidal mechanisms can be used by infiltrating immune cells, such as neutrophils, to protect compromised mucosae from microbial infection through the generation of reactive oxygen species. Unchecked, collateral damage to the surrounding tissue from neutrophil-derived reactive oxygen species can be detrimental; thus, maintenance and restitution of a breached intestinal mucosal barrier are paramount to host survival. Redox reactions and redox signalling have been studied for decades with a primary focus on contributions to disease processes. Within the past decade, an upsurge of exciting findings have implicated subtoxic levels of oxidative stress in processes such as maintenance of mucosal homeostasis, the control of protective inflammation and even regulation of tissue wound healing. Resident gut microbial communities have been shown to trigger redox signalling within the mucosa, which expresses similar but distinct enzymes to phagocytes. At the fulcrum of this delicate balance is the colonic mucosal epithelium, and emerging evidence suggests that precise control of redox signalling by these barrier-forming cells may dictate the outcome of an inflammatory event. This Review will address both the spectrum and intensity of redox activity pertaining to host–immune and host–microbiota crosstalk during homeostasis and disease processes in the gastrointestinal tract.

Mucosae are selectively permeable host surfaces, necessary for interaction with the environment and for facilitating crucial functions including gaseous exchange and nutrient absorption[1]. Protecting these surfaces from both pathogenic and commensal microorganisms, while maintaining immune homeostasis, requires the ability to rapidly and

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Author contributions

E.L.C. researched data for the article. E.L.C. and S.P.C. made substantial contributions to discussion of content, wrote the article and reviewed/edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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Review criteria

PubMed was searched from 1999 to 2017 for articles using the terms: "reactive oxygen species", "hydrogen peroxide", "hypoxia", "microbiota", "mucosa" and "epithelium" alone or in combination. Articles in English were considered on the basis of their relevance to this article's topic. The reference lists of articles were crosschecked for additional references.

potently induce danger signals when appropriate and to promptly neutralize these signals to limit collateral damage to the mucosa. The colonic mucosa consists of a single layer of epithelia derived from the crypt stem cell niche. As crypt stem cells proliferate, daughter cells migrate along the crypt axis, differentiating into specialized epithelia of either secretory or absorptive lineages[2]. Absorptive enterocytes are responsible for water reabsorption, whereas secretory epithelia are tasked with mucus and antimicrobial peptide secretion into the lumen of the gut[2,3]. These secretions provide an essential carbon source for the microbial niche, in the form of glycosylated mucins, but they also maintain a sterile margin directly adjacent to the epithelial cells to prevent inappropriate responses to resident gut microbiota[4,5].

Immune and inflammatory responses within the gastrointestinal mucosae are characterized by profound shifts in tissue metabolism. These changes include the utilization of large amounts of energy and diminished availability of oxygen (hypoxia)[6]. Such shifts in tissue metabolism result, at least in part, from recruitment of inflammatory cells, particularly neutrophils and other polymorphonuclear leukocytes (PMNs) and monocytes[7]. A particularly prominent phenotype of acute inflammatory lesions within the intestine is localized accumulation of PMNs, termed crypt abscesses. Given the large amounts of reactive oxygen species (ROS) that can be generated by activated PMNs, the crypt abscess represents a major signalling node for reduction–oxidation (redox) signalling (defined as an elicited signalling response to a particular ROS)[8]. Resident immune cells in the intestine, which include intraepithelial lymphocytes and professional antigen presenting cells (dendritic cells and macrophages), are poised as sentinels to respond to host threats such as bacterial and viral infections, but also contribute to homeostasis by immune surveillance and by promoting a regulatory immune response[9-11]. Most of these cell types — immune, epithelial and microorganism — are capable of eliciting and/or circumventing redox signalling with profound implications for mucosal homeostasis.

An important result of active inflammation in the intestinal mucosae is the localized conversion of molecular oxygen to ROS by immune cells with subsequent tissue hypoxia. At the tissue and cellular level, hypoxia induces an array of genes pivotal to adaptation to low-oxygen states (for example, those involved with glycolysis, angiogenesis or erythropoiesis). As a global regulator of oxygen homeostasis, the aβ-heterodimeric transcription factor hypoxia-inducible factor (HIF) facilitates both tissue oxygen delivery and adaptation to hypoxia[12,13]. HIF1 and HIF2 (also known as EPAS1) are members of the Per-ARNT-Sim family of basic helix–loop–helix transcription factors. HIF activation is dependent upon stabilization of an oxygen-dependent degradation domain of the HIF1 α-subunit (HIF1α) and the subsequent nuclear translocation of this subunit to form a functional complex with HIF1β and cofactors, such as CREB-binding protein (CBP) and its orthologue histone acetyltransferase p300 (REFS[12,13]). When oxygen supply exceeds demand, iron-dependent and oxygen-dependent hydroxylation of two prolines (Pro564 and Pro402) within the oxygen-dependent degradation domain of HIF1α or HIF2α initiates the association with the von Hippel–Lindau disease tumour suppressor protein and degradation of the α-subunit via ubiquitin E3 ligase proteasomal targeting[14,15]. A second hypoxic switch operates in the carboxy-terminal transactivation domain of HIF1α or HIF2α. Here, hypoxia blocks the hydroxylation of Asn803, thereby facilitating the recruitment of CBP–p300 (REF.[16]).

A unique feature of the intestinal mucosa, particularly the colon, is the juxtaposition to large numbers of microorganisms, termed the gut microbiota. Indeed, the mammalian gastrointestinal tract is home to $>10^{13}$ microorganisms, which approximates the number of eukaryotic cells constituting the human body[17]. The epithelium, a single layer of specialized absorptive and secretory cells, is all that separates this biomass from the host immune system[18]. A finely regulated relationship exists within the intestinal mucosa, whereby microorganisms, essential for host health, can also initiate and perpetuate mucosal disease[19]. Nutrient provision by microorganisms is one benefit to the host, However, in addition to aiding in digestion, microorganisms benefit the host through the local synthesis of short-chain fatty acids (SCFAs), including butyrate, propionate and acetate[20]. SCFAs can reach luminal concentrations of 130 mM in the proximal colon and function as the primary metabolic fuel for intestinal epithelial cells[20]. Reduced abundance of SCFA-producing microbial species has been associated with colonic disease, including IBD[21-23]. The low-oxygen (anaerobic) conditions that enable SCFA production place unusual metabolic demands on the colonic epithelium[24] and are enhanced during inflammation[8]. It is particularly notable that the gut microbiota are a key regulator of redox potential in the mucosa[25].

In addition to homeostatic and regulatory functions, ROS are well characterized to be produced by, and contribute to, disease processes — acutely during ischaemic damage, tissue injury and repair, and chronically in inflammatory conditions, such as IBD, and in colorectal cancer. In this Review, we provide an overview of redox reactions in the gastrointestinal tract and describe how various sources of redox-sensitive pathways contribute to the function of the healthy and diseased mammalian intestine. We will also discuss exciting new findings that highlight the contributions that different intensities of redox signalling in microbial–host crosstalk have towards maintaining homeostasis or facilitating disease processes within the gastrointestinal tract.

Redox signalling in the gut

ROS generation.

ROS constitute a major group of potent antimicrobial mediators and redox signalling factors. Both the gastrointestinal mucosa and associated immune cells are sources of free radicals, which are defined as chemical species with one or more unpaired electrons in the outermost orbital shell, making them chemically reactive[26]. The reduction and oxidation (redox) state of the gastrointestinal tract is contingent on the balance of antioxidants (for example, haem oxygenase or glutathione, a tripeptide consisting of glutamate, cysteine and glycine) and oxidants (for example, free radicals, reactive oxygen and nitrogen species). When an imbalance in redox state occurs, owing either to increased oxidants or insufficient neutralizing antioxidants, the tissue experiences oxidative stress or nitrosative stress[27]. In the gastrointestinal tract, a variety of reactive oxygen radicals, including superoxide ($O_2^{\cdot-}$) and hydroxyl ($OH^{\cdot}$), and non-radicals, including hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2), are generated by epithelial cells, endothelial cells and innate immune cells to implement mucosal defence[28] (FIG. 1). Tissue homeostasis is influenced in a variety of ways by the redox state of the tissue, including modulation of signal-transduction pathways

(for example, HIF, nuclear factor-κB (NF-κB) and nuclear factor erythroid 2-related factor 2 (NRF2))[29] that elicit adaptive gene expression to minimize bystander tissue damage. Through reduction of disulfide bonds found in many gut peptides, redox state can also modulate the activity of antimicrobial peptides involved in mucosal defence and cytokine secretion[30]. Of particular significance is the redox state of the ubiquitously expressed human β–defensin 1 (BD1). In the oxidized state, BD1 exhibits limited antimicrobial activity; however, following reduction of the disulfide bridges, BD1 alters conformation and displays an enhanced antimicrobial efficacy[31]. Indeed findings from the Wehkamp group demonstrate that the reduced form of BD1 is capable of forming net-like structures around bacteria to limit bacteria invasion[32].

Reactive nitrogen species.

Nitric oxide (NO) is a short-lived, lipophilic and freely diffusible signalling molecule synthesized by mammalian cells with a broad spectrum of activities including regulation of blood flow, immune reactions and smooth muscle contraction[33]. NO is generated by the nitric oxide synthase (NOS) enzymes, which convert L-arginine to L-citrulline, liberating NO as a by-product[34]. In the gastrointestinal tract, NO functions as an inhibitory nonadrenergic, noncholinergic neurotransmitter and smooth muscle cell relaxant via activation of guanylate cyclase[35,36]. To date, three isoforms of NOS have been cloned: neuronal NOS (nNOS; NOS1), endothelial NOS (eNOS; NOS3) and inducible NOS (iNOS; NOS2)[37-39]. Interaction of $O_2^{\cdot -}$ with NO leads to the formation of peroxynitrite ($ONOO^-$)[40]. Further reactivity of peroxynitrite leads to the generation of various other NO-derived mediators termed reactive nitrogen species (RNS), including the reactive radical compounds nitrogen dioxide ($NO_2\cdot$) and hydroxyl radical ($HO\cdot$), and nonradical dinitrogen trioxide (N_2O_3)[40]. $ONOO^-$, together with RNS, are in turn responsible for nitrosylation of protein tyrosine residues, mitochondrial energy depletion, lipid peroxidation and induction of DNA strand breaks[41]. Nitrosative and oxidative stress have been implicated in a plethora of disease states, including conditions that affect the gastrointestinal tract (namely ischaemia–reperfusion injury (IRI) and inflammatory bowel diseases)[29].

Sources of ROS.

Both exogenous and endogenous sources of ROS contribute to the overall redox state of the gastrointestinal tract. Endogenous sources contributing to ROS generation include the mitochondrial respiratory chain[42], enzymes within the mucosal epithelia and submucosal lamina propria fibroblasts and myofibroblasts, such as NADPH oxidases (NOXs), xanthine oxidase and immune-expressed cyclooxygenases, lipoxygenases and myeloperoxidase[27]. Exogenous or environmental sources of ROS can also trigger oxidative stress, such as ionizing and nonionizing radiation, chemotherapeutics, xenobiotics, heavy metals and drugs[43,44]. Generation of ROS by cancer chemotherapeutic agents is a major contributor to the toxic adverse effects associated with these compounds[44]. Cigarette smoke comprises >7,000 chemical compounds and oxidative agents, containing $>10^{14}$ free radicals per inhalation[45]. Tobacco use is known to modulate gastrointestinal diseases, and active smokers have an increased risk for colorectal cancer[46] and increased severity of Crohn's disease[47]. For reasons that are not completely clear, tobacco smoke seems to confer a somewhat protective influence to patients with ulcerative colitis[48].

Mitochondrial metabolism and ROS

Although mitochondrial ROS (mtROS) are renowned for causing cellular damage (for example, during IRI)[49], mtROS are now thought to contribute to healthy cellular function in terms of oxygen sensing, as well as disease[50]. Physiological production of mtROS occurs during oxidative phosphorylation and generation of high-energy ATP. The tricarboxylic acid cycle is tightly regulated; however, <2% of O_2 consumption results in conversion to $O_2^{\cdot-}$, whereby electrons leak out from the mitochondrial electron transport chain (ETC) and are aberrantly transferred to molecular oxygen[51]. Mitochondrial ETC complexes are capable of generating ROS at various sites. Complex I and II release $O_2^{\cdot-}$ into the mitochondrial matrix[52], whereas manganese superoxide dismutase (SOD) converts it to H_2O_2. Complex III can produce $O_2^{\cdot-}$ within the inner membrane, but it is ejected into the intermembrane space owing to a large transmembrane electrical gradient[53]. If $O_2^{\cdot-}$ generated by the mitochondrial ETC is not efficiently converted to H_2O_2, NO radicals produce $ONOO^-$, leading to subsequent irreversible nitration of proteins and enzyme inactivation[41].

Cellular stressors such as ROS and hypoxia are hallmarks of pathogen invasion but also reflect the local environmental fluctuations experienced by intestinal epithelial cells during active inflammation or infection[6]. Interest exists in autophagy as a substantial contributor to intestinal disease mechanisms, especially IBD[54]. Autophagy represents a primordial cellular degradation pathway that facilitates cell survival under conditions of metabolic stress, in which cytoplasmic targets are engulfed by a double-membrane vacuole <1 μm in diameter termed the autophagosome that is subsequently fused with lysosomes for hydrolase-mediated digestion[55]. Considerable overlap exists between cellular stimuli for selective autophagy of damaged organelles (self) and invading microorganisms (non-self)[56]. Mitophagy is a particular type of autophagy, in which mitochondria are specifically targeted for autophagic lysosomal degradation[57]. Mitophagy is a highly regulated event and some studies indicate that the mitochondrial 18 kDa translocator protein (TSPO) is central to both regulation of mtROS generation and the induction of mitophagy[58]. Interestingly, the overexpression of TSPO in animal models of IBD has revealed that TSPO localizes with epithelial mitochondria[59]. Considering the endosymbiotic theory, which postulates the ancient common origin between mitochondria and proteobacteria[60], it is curious to speculate how a pathway such as autophagy evolved to ignore functionally competent mitochondria and their proteobacterial ancestors but be triggered by invasive pathogenic organisms or damaged mitochondria.

H_2O_2 as a signalling molecule.

Oxygen radicals have a limited range of effect owing to their short-lived and highly reactive nature[61]. Specialized enzymes, such as SODs, convert oxygen radicals to the more stable and readily diffusible H_2O_2. Owing to its reduced reactivity, increased half-life and ability to induce reversible protein modification, H_2O_2 can act as a signalling molecule in its own right[61]. H_2O_2 has been demonstrated to oxidize cysteinyl thiol, induce disulfide bond formation and mediate glutathionylation of cysteine or sulfoxidation of methionine residues in numerous proteins. Such modifications can alter protein activity (increased or decreased) but also represent an important antioxidant defence mechanism[62]. In this Review, we focus

primarily on the role of H_2O_2 in mucosal–microbiota crosstalk, but it is noteworthy that other redox signalling mechanisms (for example, nitrosylation) provide important signalling cues during host–bacterial interactions[63].

Antioxidant pathways.

Regulators of the antioxidant response include enzymes that catalyse and neutralize ROS, ensuring their potent activity is short-lived to minimize collateral damage to the host tissue. Within the gastrointestinal mucosa antioxidant defence systems, SODs and glutathione peroxidase enzymes act as detoxification pathways for ROS. SODs are metal ion cofactor-requiring enzymes that catalyse the dismutation (that is, partitioning) of $O_2^{\cdot-}$ to H_2O_2 and O_2 (REF.[64]). In humans, there are three SOD isoforms: mitochondrial SOD (manganese-requiring) and cytosolic SOD and extracellular SOD (both requiring copper and zinc). Mucosal injury mediated by H_2O_2 can be mitigated by SOD activity in the gastrointestinal tract[65]. Indeed, increased SOD activity is associated with mucosal healing of human gastric ulcers, whereas reduced SOD correlates with increased ulcer severity[66].

Conversion of glutathione into oxidized glutathione is performed by the glutathione peroxidase (GPX) enzyme system. In this process, H_2O_2 is enzymatically reduced to H_2O (REF.[67]). Within the human gastrointestinal tract, expression of GPX1 is ubiquitous, but GPX2 is expressed specifically in epithelial cells[68] and is postulated to protect the mucosa from transporting luminal-derived lipid hydroperoxides[69]. Deletion of either *Gpx1* or *Gpx2* in mice had no phenotypic effect, but double-knockout mice develop spontaneous colitis[70]. Dismutation of H_2O_2 can also be achieved by the enzyme catalase, which converts $2H_2O_2$ to $2H_2O$ and O_2 (REF.[71]). Peroxiredoxins (PRDXs) are another important family of thiol-specific antioxidant enzymes, designated PRDX1–6 and encoded by six different genes (reviewed extensively elsewhere[72]). It is notable that little redundancy exists within this family of proteins, where the loss of individual PRDXs lead to numerous pathologies, including haematological disorders, tumours and increased susceptibility to diseases associated with oxidative stress[73]. Somewhat surprisingly, mice deficient in PRDX2 and PRDX6 are protected from acute colitis[74,75]. Although not completely clear, the mechanism of PRDX2-mediated protection might involve ROS-dependent stability of fork-head box protein O1 (FOXO1) and FOXP3 regulatory T cell development.

NRF2 is a crucial regulator of the antioxidant response. NRF2 forms heterodimers with small MAF proteins and binds to antioxidant response elements in the regulatory region of promoters of cytoprotective and antioxidant enzymes, regulating de novo transcription. Kelch-like ECH-associated protein 1, an adaptor subunit of cullin 3 ubiquitin ligase, regulates the function of NRF2 by acting as a redox sensor (reviewed elsewhere[76]). Thus, antioxidant pathways provide an equally important and substantial balance to redox signalling responses in the gastrointestinal tract.

Redox signalling in the immune system

Active mucosal inflammation can rapidly deplete both nutrients and oxygen in the immediate environment. For example, when activated, PMNs can increase their O_2 demand by as much as 50-fold in the generation of ROS (the so-called respiratory burst mediated by

NOX) necessary to kill microorganisms following phagocytosis[77]. By contrast, proliferating T cells only moderately increase oxygen consumption during immune responses[78]. Mucosal tissues possess both the ability to generate and attenuate redox signals; however, it is widely accepted that in the context of inflammation, the majority of radicals and reactive species are derived predominantly from the activity of resident and infiltrating immune cells, in particular, professional phagocytes of the innate immune system, such as neutrophils, monocytes, macrophages, dendritic cells and mast cells.

NADPH oxidases and ROS.

The plasma-membrane NOX family of enzymes are a group of paralogous enzymes, sharing common subunits. The complexes are made up of both membrane and cytosolic protein subunits that, upon activation, organize in the membrane to catalyse the conversion of O_2 to $O_2^{\cdot-}$ (REF.[79]). The spectrum of NOX-mediated activity ranges from potent bactericidal capacity of professional phagocytes to critical intracellular signalling in numerous cell types.

In terms of enzymatic capacity, the redox factors produced by phagocyte oxidases and peroxidases exemplify the extreme end of the redox spectrum. In addition to phagocytes expressing NOX2, fibroblasts, endothelial and epithelial cells all express enzymes that enable generation of ROS, including NOX1, NOX3, NOX4, NOX5, as well as dual oxidases DUOX1 and DUOX2 (REF.[80]). DUOX2 and NOX4 are expressed throughout the human gastrointestinal tract, whereas NOX1 expression is highest in the distal colon, where it is restricted to the cytosol, presumably to transduce intracellular signalling[28]. By comparison, DUOX2 is expressed on the apical surface of epithelia, ostensibly enabling luminal secretion of ROS[81]. Others have examined the influence of NOX1-derived or DUOX2-derived ROS on *Campylobacter jejuni* infection and discovered that ROS impaired bacterial capsule formation and virulence by altering *C. jejuni* gene expression[82].

ROS and innate immunity.

Innate immune cells, including neutrophils, macrophages and dendritic cells, represent the front line of immune surveillance and defence, and generation of ROS is a crucial microbicidal mechanism used by these cells. Activation of the NOX complex in innate immune cells elicits a rapid and potent respiratory burst[83]. Defects in phagocyte NOX function, such as in patients with chronic granulomatous disease (CGD), lead to leukocytes capable of phagocytosing but with impaired bacterial clearance[84]. The hallmark of CGD is recurrent bacterial and fungal infections. Typically, ~40% of patients with CGD develop IBD-like symptoms[85].

Following their recruitment to sites of inflammation, monocytes can polarize into either classically activated (M1) or alternatively activated (M2) macrophages, depending on the redox state and cytokine milieu of the mucosa[86]. Typically, TNF and IFNγ are accepted to elicit an M1 phenotype and T helper type 2 cytokines result in M2 polarization; however, it is also apparent that macrophage phenotypes can be mixed[87]. These differentially polarized macrophages exhibit a spectrum of functionalities. The M1 phenotype is regarded as pro-inflammatory and characterized by expression of iNOS and, consequently, these cells are an important source of RNS[88]. M2 macrophages are thought to demonstrate various activities

ranging from wound healing (release of transforming growth factor-β) to suppressing T cell function[11]. Expression of the enzyme arginase 1 by M2 macrophages depletes L-arginine, resulting in a downregulation of the T cell receptor (TCR) ζ chain[89], impairing T lymphocyte function and resulting in immunosuppression. Aside from suppressing T cell function, ROS also contribute to regulatory T cell polarization and function[90,91], but the exact molecular mechanisms have yet to be elucidated. Taken together, the net influence of ROS in macrophage polarization might promote a state of immune tolerance as it relates to regulation of T cell function.

Host–microbial interactions and ROS

The mammalian large intestine is host to trillions of bacteria, viruses and fungi, collectively termed the microbiota. A finely balanced mutualism exists within the intestinal mucosa, in which microorganisms, essential for host health, might also initiate and perpetuate mucosal disease[92]. The epithelium that lies juxtaposed to the mucosal immune system serves as a selective conduit between the host and microbial world. Recognizing that both the host and the gut microbiota (both commensals and pathogens) can generate a variety of ROS, the contribution of redox signalling to such interactions has emerged as a critical interface to host–microorganism interactions in the gut (FIG. 2).

Resident gut microbiota and ROS.

A number of studies from the Neish group have highlighted a beneficial influence of probiotic and resident microorganisms in eliciting ROS generation from epithelial sources[93] (FIG. 1). In both *Drosophila melanogaster* and mouse models, *Lactobacillus* spp. were shown to induce epithelial-derived ROS via NOX1 activity, which stimulated epithelial proliferation[94]. Subsequent studies by this group demonstrated ROS signalling dependence on the redox-sensitive transcription factor NRF2 through mechanisms that involve cytoprotection and decreased epithelial apoptosis[95]. Further studies from this group and others have elegantly implicated a role for epithelial-expressed formyl peptide receptor (FPR), responding to microbial *N*-formyl-methionine-leucine-phenylalanine, in intestinal epithelial wound healing[96,97]. This wound healing response was found to occur through oxidative inactivation of the regulatory phosphatases PTEN and PTP-PEST, with associated activation of focal adhesion kinase and paxillin[98]. Central to such mucosal wound healing responses seems to be the regulation of epithelial cell migration. For example, redox sensitive tyrosine phosphatases (for example, SE1P2 and LMW-PTP) that are expressed at the edge of wounded epithelial monolayers are crucial to the organization of focal adhesions that organize epithelial migration and wound closure[99]. Likewise, the FPR ligand annexin A1 (ANXA1) activates the generation of ROS through NOX1 and activates focal adhesion kinases necessary for wound healing and tissue resolution[98]. Loss and gain of function studies have shown that both ANXA1-null and NOX1-null mice show substantial deficits in mucosal wound healing responses and that ANXA1 delivery promotes wound healing[98]. These investigators have also demonstrated that FPR-mediated or NOX2-mediated ROS generation in mice at local intestinal tissue sites selects for mucus-resident microorganisms, including *Akkermansia muciniphila*, that accelerate epithelial wound healing in an intestinal

epithelial NOX1-dependent fashion[100]. Although not completely clear, this wound healing response seems to require ERK phosphorylation within the epithelium.

ROS and pathogen niche expansion.

Similar to resident gut microorganisms, opportunistic pathogens also use redox reactions to subvert host defences and establish a niche. One of the most studied in this regard is the invasive enteric pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium. This pathogen is associated with acute gastrointestinal inflammation and diarrhoea, and elicits neutrophil chemotaxis into the mucosa[101]. Invasion is achieved through two type III secretion systems that facilitate *S.* Typhimurium to enter and persist inside intestinal epithelial cells and mucosal macrophages[101]. Before invasion, *S.* Typhimurium must outcompete the resident gut microbiota. Some studies indicate that inflammation amplifies proliferation of luminal *S.* Typhimurium, enabling it to overgrow other microorganisms[102]. In one report, the Bäumler group demonstrated that inflammation-induced intestinal ROS reacted with luminal thiosulfate to form a new respiratory electron acceptor, tetrathionate[103]. Moreover, *S.* Typhimurium express genes to enable utilization of tetrathionate as an electron acceptor that enables the pathogen to use respiration to outcompete fermenting microorganisms and establish a niche[103]. The authors subsequently demonstrated that this tetrathionate-enabled respiration provided another growth advantage to *S.* Typhimurium, enabling the utilization of epithelial-derived ethanolamine under anaerobic conditions[104].

ROS and pathogen niche restriction.

The role of H_2O_2 secretion into the lumen of the gut is poorly understood, but several roles have been proposed. Some studies suggest a pro-inflammatory function for DUOX-derived H_2O_2, acting as a chemotactic signal for neutrophils in a zebrafish wound healing model[105] and a mouse allergic airway model[106]. Other findings suggest that apical secretion of H_2O_2 into the lumen of the gut is implicated in restricting *Helicobacter felis* colonization in mice through increased bacterial oxidative stress[107]. Another study examined the influence of NOX1-derived or DUOX2-derived ROS on *C. jejuni* infection and discovered that ROS impaired bacterial capsule formation and virulence by altering *C. jejuni* gene expression[82]. During *Citrobacter rodentium* infection in wild-type mice, the Knaus group discovered that NOX1 regulates DUOX2 expression in the mucosal epithelium, with a resultant decrease in both $O_2^{\cdot-}$ and H_2O_2 production[108]. An unexpected but intriguing finding from this study was that ablation of epithelium-derived ROS, using an epithelium-restricted CYBA-deficient mouse (absence of the obligatory NOX dimerization partner), led to protection from colitis induced by *C. rodentium*. The authors attribute their findings to an altered gut microbiota with an expansion in H_2O_2-producing lactobacilli, which exert antimicrobial activity through release of urease, lactic acid and H_2O_2 (REF.[109]). Pircalabioru et al.[108] demonstrated through the use of catalase to degrade H_2O_2 that H_2O_2-producing lactobacilli were responsible for attenuating *C. rodentium* virulence factors. Other findings to support H_2O_2 exerting an antimicrobial function include disruption of microbial intracellular signalling, which affects antioxidant defence and polysaccharide biosynthesis[110]. In the human body, L-amino acids are essential for protein synthesis; however, D-amino acids function in necessary non-ribosome-based roles[111]. Bacteria synthesize and secrete distinct D-amino acids into the lumen of the colon[112], and the Waldor group demonstrated that microbiota-derived D-amino

acids upregulate expression of the host epithelial-expressed enzyme, D-amino acid oxidase, which is secreted into the lumen. Oxidative deamination of D-amino acids by D-amino acid oxidase yields H_2O_2 as a by-product and protects from *Vibrio cholerae* pathogenicity[113].

Consequences of redox signalling

Redox-sensitive signalling pathways are often limited by the availability of extracellular and intracellular oxygen[114]. Despite this understanding, ROS generation can occur at surprisingly low oxygen tensions. The neutrophil NOX, for example, is fully functional at ambient oxygen concentrations as low as 1%[77]. Such observations highlight the importance of differentiating oxygen and ROS diffusion within the tissue microenvironment, as well as the variability of oxygen availability within individual tissues. These differences often determine end-point tissue function and define the adaptability of tissues to hypoxic stress[115].

Colonic tissue oxygen metabolism.

The partial pressure of oxygen (pO_2) at sea level is ~145 mmHg, and the alveoli of healthy lungs experience a pO_2 of ~110 mmHg (REF.[116]). The lumen of the colon is virtually anoxic, mainly due to the microbial biomass[117], and colonic epithelia adjacent to the lumen experience and withstand a pO_2 < 10 mmHg (REF.[118]). Thus, it might be surprising to discover that epithelial stem cells at the crypt base are highly oxygenated (experiencing a pO_2 of ~100 mmHg)[92]. Such differences are compounded by epithelial metabolism and the arrangement of the microvasculature network with countercurrent blood flow dynamics in each villous structure[92]. Epithelia adjacent to the lumen effectively exist in a state of physiological hypoxia[119] and are uniquely susceptible to changes in the redox state. Experimental use of oxygen-sensitive nitroimidazole compounds, such as pimonidazole, has enabled visualization of hypoxia in these tissues both basally and during inflammation[120]. It is notable that these dyes are neither dependent on redox enzymes nor changed by NADH and NADPH levels[121]. This technology, coupled with immunostaining, has been used to visualize oxygenated (or lack thereof) regions of large solid tumours in rats[122]. It is notable that pimonidazole adducts might serve as a more reliable marker than staining for HIF1, as it is retained in chronically hypoxic cells[123]. Such physiological hypoxia (FIG. 1) is reversible by oxygenation of the colonic lumen (for example, using oxygenated perfluorodecalin)[124].

Adaptive responses to hypoxia involve the stabilization of HIF, a master regulator of oxygen homeostasis[12]. Prolyl hydroxylase (PHD) enzymes continually utilize cellular O_2, in conjunction with 2-oxoglutarate and iron, to target HIF for hydroxylation and subsequent ubiquitylation and degradation[125]. Various factors in addition to limited O_2 availability influence the activity of the PHD enzymes and HIF stabilization. Among them, H_2O_2 has been demonstrated to stabilize HIF via inhibition of the PHD2 enzyme[126]. Transcriptional activity of the HIF transcription factors regulates the genes involved in adaptive responses to hypoxia, the most widely acknowledged include angiogenesis-related and glycolysis-related genes (for example, *VEGFA, NOS2, SLC2A1* and *PGK1*). Less well characterized, but rapidly increasing in number, are genes with associated mucosa-protective functions that enable colonic epithelial cells to restore impaired barrier function (for example, *TFF3,*

MUC3A, CLDN1 and *ABCB1*)[118]. Original studies using genetic loss and gain of intestinal epithelial *Hif1a* expression in mice revealed a protective role for HIF in chemically-induced colitis models that corresponded to mucosal barrier protection[120]. Studies with cultured intestinal epithelial cells exposed to conditions that activate HIF have identified the regulation of a number of barrier-related protective genes[127] that have now been validated in animal models of colitis[120,128-132] and in human-derived colonic tissue[8,133-135]. The intestinal epithelial barrier proteins encoded by HIF target genes include those that localize to the apical surface of polarized epithelia, including mucins and mucin modifiers (for example, intestinal trefoil factor), tight junction proteins (claudin 1), antimicrobial peptides and proteins important for xenobiotic clearance[118]. Each of these components is a direct transcriptional target of HIF and contributes fundamentally to the 3D intestinal tissue architecture that forms an intact barrier during homeostasis.

Induction of epithelial HIF.

Following recruitment of immune cells to the mucosa, for instance following induction of experimental colitis, hypoxia extends deeper into tissue[120], a phenomenon termed inflammatory hypoxia[136] (FIG. 1). A study by Campbell et al.[8], demonstrated that during experimental murine colitis, neutrophil influx was primarily responsible for inflammatory hypoxia. By use of a combination of neutrophil antibody depletion, hypoxia-reporter mice and NOX2-deficient ($Gp91^{phox-/-}$) mice, the authors demonstrated that functional NOX2 not only disseminated mucosal hypoxia but also stabilized HIF within the intestinal epithelium. This HIF signature within the epithelium resulted in an adaptive transcriptional response, that the authors coined transcriptional imprinting[8]. Biopsy samples from patients with ulcerative colitis with evidence of crypt abscess formation — a pathological hallmark of transmigrated neutrophils within the lumen of the crypt — revealed induction of HIF (monitored by increases in the HIF-target gene *SLC2A1*). However, it is unclear from these studies if HIF stabilization is due to depletion of oxygen or generation of $O_2^{\cdot-}$ or H_2O_2, as all are capable of inhibiting the PHD enzymes[8,79].

Another means to stabilize HIF by inhibition of PHD enzyme function is via depletion of another crucial cofactor: iron. Some findings indicate that certain microorganisms, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, can stabilize HIF in lung epithelia via secretion of low-molecular-weight, high-affinity iron chelators, termed siderophores[137,138]. Presumably, these factors function to chelate iron bound within the active site of the PHD enzymes, although this has not been shown conclusively. Fermenting microbiota have also been shown to stabilize HIF in the colon, via SCFA release, particularly via butyrate production[139]. Butyrate is used as the preferred enterocyte fuel source, oxidizing butyrate to CO_2 (REF.[140]). The net effect is epithelial hypoxia owing to increased oxygen consumption, likely resulting in PHD enzyme inhibition to facilitate HIF stabilization[139]. Indeed, a study from the Bäumler group in 2016 demonstrated that streptomycin-treated mice exhibited a decline in butyrate-producing clostridia, which led to increased oxygenation of the mucosal epithelium, enabling enhanced *Salmonella* expansion[141].

Inflammasome activation.

The NLRP3 inflammasome is an intracellular multiprotein complex involved in perceived cellular danger response[142]. Pathogen-associated molecular patterns and host-derived danger-associated molecular patterns can trigger inflammasome activation[143]. Stimulation of NLRP3 leads to assembly of this inflammasome complex and, ultimately, to caspase-1 activation and downstream cleavage of pro-inflammatory cytokines IL-1β and IL-18 (REF. [144]). The role of IL-1β is widely studied in autoimmune diseases; however, in gastrointestinal inflammation, its involvement is not fully understood. Studies using mouse models of chronic colitis have demonstrated a role of IL-1β in accumulation of IL-17A-secreting $CD4^+$ T helper 17 cells[145]. One study in 2017 by Neudecker et al.[146] implicated CC-chemokine receptor 2 $(CCR2)^+$ monocytes and NLRP3 activity leading to IL-1β production in the pathogenesis of acute colitis in mice. Surprisingly, mice lacking NLRP3 are hypersensitive to experimental colitis, displaying exacerbated immune infiltration and epithelial damage, primarily due to a loss of IL-18 (REF.[147]). Despite the intense interest in the field, relatively little is known about how the NLRP3 inflammasome is regulated at a molecular level. Some studies suggest that autophagy can negatively regulate the NLRP3 inflammasome[148]. Conversely, activation of ROS, ostensibly by NOXs[149], has been shown to stimulate the NLRP3 inflammasome. However, patients with CGD with NADPH-deficient macrophages display normal inflammasome activation in several studies[150,151], implicating other stimuli aside from NOX-derived ROS. Another abundant source of cellular ROS are mitochondria, which release ROS in response to elevated metabolism, hypoxia or membrane damage[152]. The Tschopp group demonstrated that inhibition of mitophagy (encapsulation and degradation of old or damaged mitochondria via cellular autophagic machinery) results in the accumulation of damaged ROS-generating mitochondria, which leads to NLRP3 inflammasome activation[153]. The authors subsequently demonstrated that both ROS generation and NLRP3 activation were suppressed when mitochondrial activity was disrupted by blockade of the voltage-dependent anion channel. These findings suggest that the NLRP3 inflammasome can perceive mitochondrial dysfunction[153].

Goblet cell mucus secretion.

Goblet cells are specialized epithelial cells that protect the barrier from microbial invasion by secretion of a mucus hydrogel[154]. The principal components of mucus are large mucin peptides arranged in polymeric structures. Following translation, mucins undergo extensive *N*-linked and *O*-linked glycosylation modifications and are packaged into vesicles[4]. Goblet-cell-derived glycosylated mucins provide a major carbon source for the gut microbiota (reviewed elsewhere[5]). Interest has centred on understanding the regulation of mucin packaging and secretion at baseline and in response to microorganism detection[155], which has led to the suggestion that goblet cells are actually an unappreciated and distinct innate immune cell[4,156]. Another secretory epithelial lineage, Paneth cells, which are tasked with antimicrobial peptide secretion and defence of the intestinal stem cell niche, rely on autophagy to organize secretory granules[157]. By contrast, autophagy-compromised goblet cells displayed normal mucin packaging into granules[158]. However, a combination of autophagy and NOX-derived ROS were found to be essential for mucin release by goblet cells (FIG. 2). Patel et al.[158] demonstrated that amphisome-like vesicles form in goblet cells following autophagosome and endosome fusion and these specialized organelles regulate

mucin secretion. It was subsequently demonstrated that the NLRP6 inflammasome is crucial for promoting goblet cell mucin release in response to proximity with microorganisms[159]. In 2016, the Hansson group proposed the existence of a 'sentinel' goblet cell[160] based on proximity to the crypt entrance. This sentinel goblet cell nonspecifically endocytoses and responds to Toll-like receptor ligands, stimulating NOX1-dependent or DUOX2-dependent ROS production, through downstream activation of the NLRP6 inflammasome. Moreover, via intercellular gap junctions, signals are transduced down the crypt axis to elicit mucin secretion from neighbouring goblet cells[160].

Secretion of additional mucins in response to detection of microbial proximity is obviously one approach to repel a microbial onslaught, but goblet cell hyperplasia is an alternative mechanism. As mucus erosion and goblet cell depletion are pathological hallmarks of ulcerative colitis[161], repletion of both goblet cells and their mucin granule contents is necessary for epithelial barrier restitution. As mentioned previously, IL-18 secretion is stimulated by ROS-mediated inflammasome activation[144], where some findings reveal distinct and opposing roles for IL-18 and IL-22 signalling in regulating goblet cell homeostasis. For example, the Flavell group demonstrated, using various intestinal-epithelial-specific knockout mice to target IL-18 signalling, that excess IL-18 promotes goblet cell depletion and colitis[162]. Moreover, IL-18 seems to suppress goblet cell differentiation markers. Contrastingly, immune-cell-derived IL-22 has well-recognized protective mucosal effects via promoting stem cell differentiation, mucin synthesis (mucin 2), antimicrobial peptide (REG3γ) and goblet cell function (*Fut2* expression)[163-165]. The recently characterized type 3 innate lymphoid cells (ILC3s) are the major source of IL-22 within the intestinal mucosa[166]. In fact, during *Toxoplasma gondii* infection, ILCs and T cells required epithelial-derived inflammasome-processed IL-18 in order to release IL-22 (REF.[167]). Thus, a combination of redox signalling, inflammasome activity and immune crosstalk might hold the key to homeostasis between IL-18 and IL-22 signalling and indeed mucosal-microbiota homeostasis. Moreover, IL-1β can both induce activation of ILC3s and contribute to plasticity (in concert with other factors, including retinoic acid) and reprogramming of ILC1s and ex-ILC3s to ILC3s[168].

Redox signalling in intestinal disorders

Ischaemia–reperfusion injury.

Ischaemia is defined as insufficient blood flow to tissues, resulting in disruption of cell function and ultimately necrosis. Various tissue insults can lead to intestinal ischaemia, including trauma, stroke and atherosclerosis, and reperfusion (restitution of blood supply) following ischaemia can result in aggravated tissue damage. The intestine is particularly sensitive to IRI[169]. Ischaemia rapidly induces expression of cyclooxygenase (COX) and accumulation of cells expressing lipoxygenase enzymes, which are responsible for generating pro-inflammatory eicosanoids from membrane-liberated arachidonic acid, such as prostaglandins and leukotrienes[170]. Constitutively expressed COX1 and the inducible isoform COX2 are responsible for catalysing the conversion of arachidonic acid to prostaglandins[171]. The primary prostaglandin studied in this context is prostaglandin E_2 (REF.[172]), which elicits a bifunctional influence on the intestinal mucosa, promoting injury

via vasodilatory influences on the endothelium but simultaneously conferring cytoprotection to the intestinal epithelium[173]. Infiltrating leukocytes, recruited by endothelial-derived leukotriene B_4, facilitate neutrophil adhesion, activation and degranulation[174]. Following reperfusion, a necessary substrate (O_2) becomes available to enable the de novo synthesis of an 'eicosanoid storm', in which bioactive lipids of the eicosanoid family become substantially amplified in their production[175]. This combination of lipid mediator generation, complement activation[176] and neutrophil accumulation in the tissue milieu results in another consequence of IRI: lipid peroxidation[177] (the oxidative degradation of lipids that result in plasma membrane and organelle damage). ROS generated by re-oxygenated neutrophils have long been recognized as instigators of lipid peroxidation in intestinal IRI, resulting in barrier disruption[49,135,169,177]. Indeed, treatment with SOD in a murine intestinal IRI model limits the contribution of ROS to both lipid peroxidation and mucosal permeability[177]. Experimental strategies to circumvent the deleterious exaggerated inflammation and resultant tissue damage occurring in IRI mostly hinge on reducing neutrophil recruitment signals and leukocyte activation[178]. The anti-inflammatory influences of carbon monoxide (CO), derived from endogenous haem oxygenase, might be a promising therapeutic strategy to attenuate damage from IRI[179]. Multiple lines of evidence have revealed that the activation of haem oxygenase effectively promotes cytoprotection and inhibits the pro-inflammatory signatures elicited by multiple cell types during intestinal IRI[179]. Strategies to induce haem oxygenase and CO release are in development and include haem oxygenase 1 fusion proteins, small molecule haem oxygenase inducers, bilirubin, glutamine and inhaled CO[179].

Ischaemic preconditioning.

Aside from leukocyte-derived sources of ROS, the mucosa itself can substantially contribute to redox-mediated damage during intestinal ischaemia. High concentrations of ATP are released extracellularly during ischaemia, which are ultimately catabolized to hypoxanthine[180]. Concomitantly, ischaemic stress results in the conversion of xanthine dehydrogenase to xanthine oxide[181]. Following tissue reperfusion, the combination of hypoxanthine, xanthine oxide and newly available O_2 yields additional sources of tissue $O_2^{\cdot-}$ (REF.[182]). Although some limited therapeutic success has arisen from scavenging ROS or targeting inflammatory mediators, one of the more promising strategies to reduce IRI is ischaemic preconditioning (or hypoxic preconditioning), whereby cells or tissues are exposed to brief and intermittent periods of non-lethal ischaemia. Such treatments have been shown to protect organs that experience a major ischaemic event, which is best studied in the heart[183]. The mechanisms involved in ischaemic preconditioning are complex but ultimately result in reduced pro-inflammatory factors, decreased lipid peroxidation and elevated levels of natural antioxidants including glutathione, SOD and haem oxygenase 1 (REF.[184]). Khoury et al.[185] identified extracellular adenosine released by hypoxic preconditioned intestinal epithelia as the major anti-inflammatory factor responsible for hypoxic preconditioning. This protective role of adenosine in ischaemic preconditioning corresponded with the inhibition of NF-κB via deneddylation (where NEDD8 is removed from a conjugated protein) of cullin 1, a component of the proteasomal degradation pathway important in the activation of NF-κB[186]. It was shown that adenosine might regulate HIF through similar mechanisms; for example, a small molecule deneddylator of the cullin

family proteins has become commercially available. This compound, MLN4924, is an AMP analogue that functionally inhibits NEDD8-activating enzyme and results in the deneddylation of cullin 1 and cullin 2 (REFS[187,188]) and has proven to be a potent HIF stabilizer in cultured cells[189]. In this regard, HIF might function to promote tissue ischaemic preconditioning, which has been shown in some studies[13], and small molecule stabilizers of HIF (especially PHD inhibitors) show promise in protection from damage associated with IRI[190].

Extracellular adenosine is derived from the enzymatic degradation of ATP via the action of surface apyrases (for example, CD39 (also known as NTPDase 1)) and ecto-5′-nucleotidase (CD73)[191]. CD73 expression is increased on intestinal epithelia during hypoxia in a HIF-dependent manner, resulting in increased extracellular adenosine accumulation[127]. Moreover, HIF stabilization in hypoxia was also demonstrated to decrease expression of the equilibrative nucleoside transporter 1 and 2, resulting in reduced uptake of extracellular adenosine providing more available for extracellular signalling[192]. Extracellular adenosine signals through activation of any of four surface G-protein-coupled receptors. Activation of the adenosine receptors A1 or A3 results in decreased intracellular cAMP levels ($G_{\alpha i}$-coupled), whereas adenosine binding to the high-affinity adenosine receptor A2a (A2AAR) or the low affinity adenosine receptor A2b (A2BAR) is associated with elevation of cAMP ($G_{\alpha s}$-coupled)[193]. The predominant receptor-mediated signalling associated with intestinal epithelial cells is A2BAR, and the crystal structure of agonist-bound and antagonist-bound A2AAR has been resolved[194]. The majority of evidence suggests that the induction of A2BAR by HIF translates to a strong anti-inflammatory phenotype in the intestinal mucosa, at least in part associated with barrier protection[195,196]. These studies have shown important protective roles for A2BAR in experimental colitis[195] and intestinal IRI[197,198] that results in diminished inflammation[199].

A number of sources of nucleotides exist in inflamed and ischaemic tissue. Many cell types actively release nucleotides, particularly in the form of ATP or ADP[193]. Programmed cell death (apoptosis) is associated with the generation of large amounts of ATP during ischaemia and inflammation. The ATP released by apoptotic cells has been demonstrated to function as a ‘find-me’ signal to promote phagocytic clearance during inflammatory resolution[200]. Other studies have shown that inflammatory cells, such as neutrophils, can release ATP in an active manner though connexin 43 (also known as GJA1) hemichannels[201,202]. Platelets release nucleotides at high concentration upon activation and are also an important source of extracellular nucleotides during ischaemia. In the intestinal mucosa, for example, platelets and neutrophils have been shown to co-migrate across the epithelium and into the lumen in the formation of crypt abscesses[203]. As originally described by Madara et al.[204], this local generation of luminal nucleotides results in adenosine-mediated activation of electrogenic chloride secretion and associated water movement into the intestinal lumen. This fluid transport process provides an important flushing of the mucosal surface during ongoing inflammation.

Role of ROS in IBD.

IBD includes ulcerative colitis and Crohn's disease and is characterized as a chronic inflammatory condition of the gastrointestinal mucosae in susceptible individuals[54]. Ulcerative colitis and Crohn's disease exhibit distinct pathophysiology in terms of effector immune functions. Common features of IBD include abdominal pain and diarrhoea, and susceptibility to IBD is dictated by a combination of genetic, environmental and microbial risk factors[54]. The microenvironment of active IBD lesions is considered to be strongly redox active, in which ROS are considered to play an important part in both inflammatory signalling and in bystander damage to surrounding tissue[29].

Does excessive ROS or insufficient ROS contribute to IBD? Evidence exists to support both excessive ROS and insufficient ROS as contributing to IBD. Considering the number and functional diversity of susceptibility genes in IBD identified by genome-wide association studies, it is likely that the answer to this question depends on the individual combination of aetiological factors and not merely the diagnosis of ulcerative colitis versus Crohn's disease. For instance, a study of 157 patients with CGD profiled IBD risk alleles among this cohort and concluded that defective $O_2^{\cdot-}$ generation in CGD is a major risk factor for IBD[205].

As alluded to earlier, the majority of patients with CGD develop IBD-like symptoms[85]. A potentially confounding issue for research in this field is the mouse models used to address the roles of phagocyte-derived ROS versus ROS from mucosal sources. Campbell et al.[8], using a 2,4,6-trinitrobenzenesulfonic acid (TNBS) model of colitis, demonstrated that *Nox2*$^{-/-}$ mice develop substantially more severe colitis, reflected by increased weight loss, increased intestinal permeability and the failure to resolve ongoing inflammation compared with wild-type control mice. Conversely, Bao et al.[206] used the same mice in a dextran sulfate sodium (DSS) model of colitis and found no difference in weight loss or disease severity compared with wild-type controls. They concluded that less tissue damage was associated with decreased oxidative burst, though no evidence was provided for increased ROS-mediated damage. A possible explanation for the discrepancy between these studies is in the nature of the models used to ascertain the relative importance of phagocyte NOX. DSS models of colitis rely on denudation of epithelial cells, beginning with erosion of apical mucus, apoptosis of epithelia and resulting innate immune infiltrate[207]. It could be argued that, under such circumstances, phagocyte-derived ROS might not be contributing to denudation of colonic epithelia; thus, only genes or therapeutic intervention that influence epithelial viability or turnover will have an appreciable effect. By contrast, TNBS models involve pre-sensitizing the host immune system to haptenized microbial antigens, with subsequent colonic exposure to the haptenizing agent[207]. DSS results in progressive tissue damage, extending proximally from the rectum, and incremental loss of body weight over the course of the experiment. TNBS-treated animals lose and regain weight rapidly over time and tend to exhibit skip lesions with relatively intact epithelia[207]. Moreover, immune infiltrates and inflammatory mediators differ substantially between the models[207]. As such, the DSS model represents a wound model, whereas the TNBS model represents an acute-to-chronic inflammation and resolution model. Thus, it is conceivable that *Nox2*$^{-/-}$ mice do not exhibit enhanced mucosal wounding but rather fail to resolve inflammatory insults.

Despite the dependence of IBD on genetic susceptibility and observed chronic adaptive immune responses, numerous aspects of disease progression are comparable between IBD and IRI. For instance, pro-inflammatory mediators such as TNF and prostaglandin E_2 are implicated in both IRI and Crohn's disease[49,208]. Involvement of neutrophils, monocytes and leukocyte-derived ROS have been implicated in both ulcerative colitis and IRI in the colon and intestine[49,209]. Similarly, epithelial barrier disruption and enhanced microbial translocation are features of both IBD and IRI[49,210]. Studies have also suggested that shifts in the gut microbiome (dysbiosis) might contribute to both IRI and IBD[49,211]. One important caveat to this understanding is the observation that antibody-mediated neutrophil depletion strategies in intestinal IRI models do not seem to influence injury end points[212], whereas neutrophil depletion substantially enhances tissue damage in multiple colitis models[213]. Another common feature between IBD and IRI models is that accumulation of extracellular ATP in colitis models has been demonstrated to contribute to the inflammatory process, in part via stimulation of the $P2X_7$ receptor[214]. Also similar to ischaemic preconditioning is that extracellular adenosine seems to confer mucosal protection during colitis, principally via A2BAR signalling. Indeed, murine models of whole-body and conditional epithelial deletion of A2BAR results in more severe DSS-induced colitis associated with decreased barrier function and diminished mucosal wound healing[195,196].

ROS-induced collateral tissue damage.

Substantial evidence exists that collateral tissue damage, the bystander effect, might result from increased oxidative stress associated with active intestinal inflammation[215]. Implications of excessive ROS-mediated tissue damage in the gastrointestinal tract include alterations of absorptive function, barrier dysfunction and dysmotility[216]. Numerous studies have, for example, indicated malabsorption of nutrients in the intestine following IRI and in IBD[217,218]. By contrast, colonic epithelia are tasked predominantly with the re-uptake of water from the faecal stream, so disruption to colonic absorption in IBD manifests as diarrhoea[219]. Extensive tissue damage from excessive ROS (for example, via lipid peroxidation or protein chlorination of mucosal barrier proteins) and from immune mediators, such as TNF and IFNγ, increases mucosal permeability by modulating tight junction function[220] (FIG. 3). One well-documented mechanism of barrier disruption is via induction of so-called 'leaky' claudin tight junction proteins, such as claudin 2 and claudin 5 (REF.[221]). Under such conditions, so-called claudin switching occurs in which physiologically distinct tight junctions, defined by their constellation of claudin components, determine the structural integrity of the epithelial barrier[221]. It is also notable that increases in vascular permeability might precede increases in epithelial permeability during active mucosal inflammation. Tolstanova et al.[222] used four murine models of colitis to demonstrate that early endothelial damage resulted in perivascular oedema and epithelial hypoxia, which contributed to the stabilization of HIF within the mucosa. Evidence for early ROS-mediated endothelial damage were demonstrated at the level of electron microscopy and were consistent in genetic models of colitis, including *Il10*$^{-/-}$ and *Gnai2*$^{-/-}$ animals (REF.[222]). Finally, it is likely that gut motility is affected by redox-sensitive mechanisms. For example, Brown et al.[223] showed that enteric neuron death during active colitis was mediated by NO derived from glial cells. This neurotoxic activity was driven by NO influences on connexin 43 activity. Moreover, exposure of submucosal smooth muscle cells

to microbial lipopolysaccharide probably contributes to dysmotility through the generation of large amounts of ROS and RNS[224]. Taken together, these studies implicate collateral tissue damage associated with oxidative stress in active intestinal inflammation.

Conclusions

The gastrointestinal tract represents a particularly austere environment for redox-sensitive signalling. The combination of multiple sources of ROS or RNS in the setting of trillions of microorganisms requires the presence of important gatekeeper mechanisms to avoid the potential chaos that could occur during active inflammation. Just as important is the need to maintain a well-poised antimicrobial environment, in large part driven by epithelial and leukocyte-derived oxygen radicals. It is notable that the profound differences in local O_2 tension within mucosal tissues and substantial increases in energy demand during inflammation provide a unique setting to understand tissue metabolism under stress. Of particular interest is the metabolic shift toward hypoxia and the associated stabilization of HIF target pathways that associates with tissue barrier function, wound healing, autophagy and inflammation resolution. Redox signals derived from immune cells, parenchymal cells (epithelia, endothelia and fibroblasts), as well as the gut microbiota, are coupled with the differing potencies, toxicities and half-lives of the redox products produced locally that require tight control for tissue homeostasis. Studies in vitro and in vivo have provided new insights toward a better understanding of productive inflammatory responses and mechanisms that promote inflammatory resolution. Also relevant is the shift in tissue redox potential that mediates collateral tissue damage and end-point organ function. A better understanding of the basic molecular signals, transcriptional programmes and the environmental cues that regulate mucosal redox state (BOX 1) are likely to provide new insight into the development of novel therapies for diseases such as IBD.

Acknowledgements

E.L.C. is supported by NIH grant DK103639, and S.P.C. is supported by NIH grants DK50189, DK104713, DK095491, DK103712 and a Merit Award from the Veterans Administration.

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Key points

- Immune cells, microorganisms and the epithelium all generate and respond to redox signals in the colonic mucosa during homeostasis and in disease.
- Redox signals, particularly H_2O_2, are generated by the host and the gut microbiota to impede overgrowth of opportunistic pathogens; similarly, certain pathogens utilize these systems to subvert host defences.
- Host responses to reactive oxygen species (ROS) produced in situ and hypoxia act in concert and opposition to regulate homeostasis in the gut.
- Host–immune and host–microbiota crosstalk can both contribute to excessive ROS production, participating in collateral damage at the tissue level.

Box 1 |

Knowledge gaps and future research directions

- Which host metabolic factors control the redox state threshold and under what conditions are they generated?
- Could microbiota-derived factors that influence redox state be enriched to benefit the host in health or during disease?
- Does the low oxygen partial pressure environment of the gut provide an opportunity for drug targeting and/or drug delivery?
- How does overall tissue redox state influence acute inflammatory resolution versus progression toward chronic inflammation?
- Is innate immunity more amenable to therapeutic targeting than adaptive immunity, or vice versa?
- Is pharmacological stabilization of hypoxia-inducible factor (for example, via prolyl hydroxylase inhibition) a viable therapeutic option for mucosal disease?
- Which pharmacological approaches best mimic ischaemic preconditioning and under which circumstances might these approaches benefit the host?
- For therapeutic targeting of redox pathways, how might we maximize the beneficial influence of redox signalling and minimize bystander tissue damage?

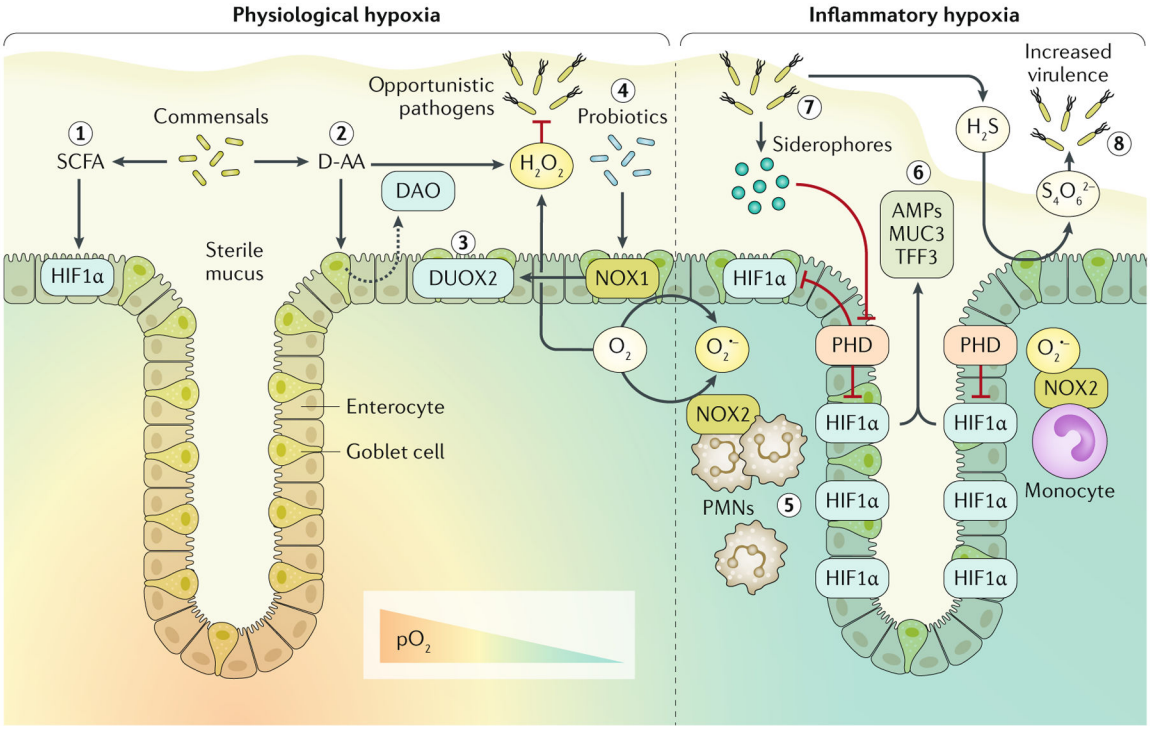

Fig. 1 |. Host-microbial redox signalling during hypoxia.

Enzymatic utilization of molecular oxygen (O_2) within the intestinal mucosa facilitates redox signalling and results in both spatial and dynamic patterns of O_2 availability. In the healthy intestinal mucosa, a steep O_2 gradient exists between the highly vascularized mucosa and the anoxic lumen. Thus, cells within the crypt stem cell niche normally experience higher partial pressures of oxygen (pO_2; ~100 mmHg) than the luminal-facing epithelia (<10 mmHg), which are known to normally experience hypoxia at homeostasis. This physiological hypoxia is experienced by epithelia adjacent to the lumen and results in stabilization of hypoxia-inducible factor (HIF). Gut microbiota secreting short chain fatty acids (SCFAs), particularly butyrate, contribute to this physiological hypoxia and associated stabilization of HIF1α through increased oxidative phosphorylation (step 1). Luminal redox signalling initiated by resident microorganisms releasing D-amino acids (D-AA) stimulates the epithelium to secrete D-AA oxidase (DAO) into the lumen, which subsequently yields hydrogen peroxide (H_2O_2) (step 2). Apical expression of epithelial dual oxidase 2 (DUOX2) probably results in luminal secretion of H_2O_2, which contributes to limiting opportunistic pathogen niche expansion (step 3). Probiotic lactobacilli upregulate epithelial NADPH oxidase 1 (NOX1) expression, which in turn induces DUOX2 (step4). Epithelial-expressed NOX1 and DUOX2, utilizing microenvironmental O_2, generate oxygen radicals to further contribute to luminal release of H_2O_2. During inflammatory hypoxia, infiltrating polymorphonuclear leukocytes (PMNs) and monocytes expressing NOX2 generate superoxide ($O_2^{\cdot-}$), resulting in inhibition of prolyl hydroxylase enzymes (PHD) and stabilization of HIF deep into the crypt (step 5). HIF transcriptional activity induces expression of barrier protective factors such as antimicrobial peptides (AMPs), mucin 3 (MUC3) and trefoil factor 3 (TFF3) (step 6). Certain opportunistic pathogens release siderophores, sequestering iron and inhibiting PHD (step 7). Sulfur metabolism of the

mucosa can be hijacked by opportunistic pathogens. Hydrogen sulfide (H_2S) is routinely detoxified to thiosulfate; however, high levels of reactive oxygen species within the mucosa can result in tetrathionate ($S_4O_6^{2-}$) generation, which can be utilized by *Salmonella* serotypes to provide a competitive advantage (step 8).

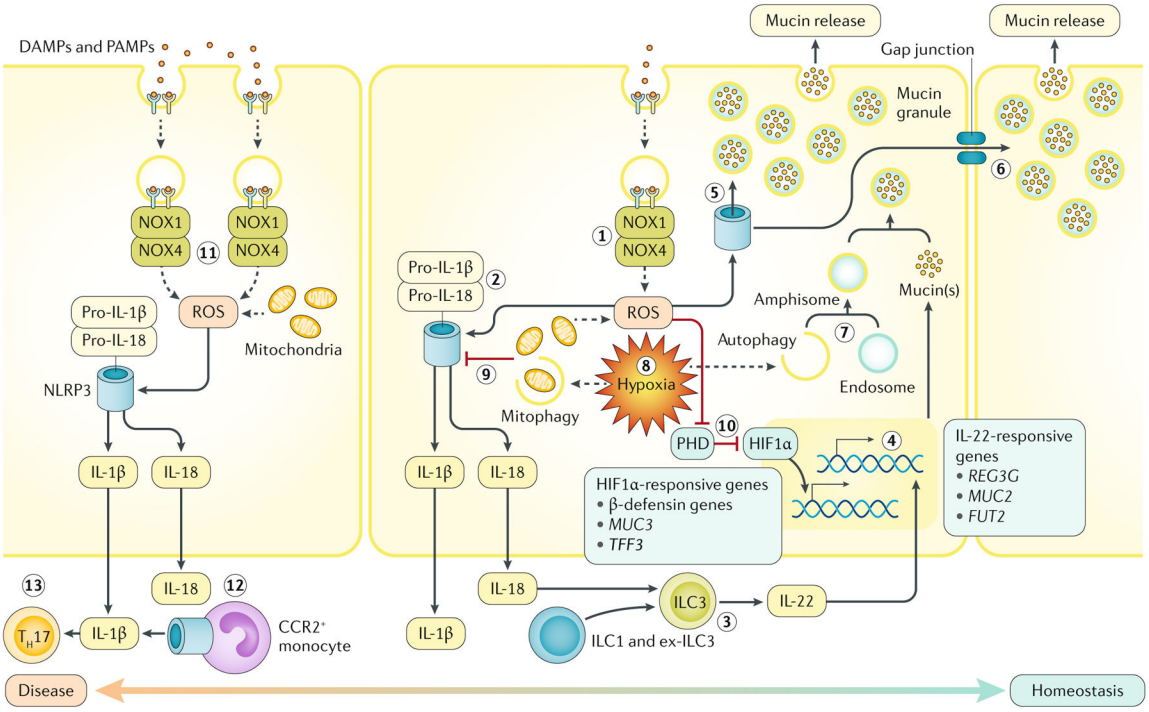

Fig. 2 | Host redox–hypoxia crosstalk in the gastrointestinal mucosa.

The two major sources of endogenous reactive oxygen species (ROS) within the intestinal epithelium originate from mitochondria and NADPH oxidase 1 (NOX1) or NOX4 (step 1). In response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), epithelia recruit and activate the NOX1–NOX4 complex, stimulating superoxide and hydrogen peroxide generation (sources of ROS). Both enzymatic and mitochondria-derived ROS can trigger the activity of epithelial inflammasomes. In colonic epithelia, ROS-stimulated NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome assembly leads to IL-18 (and IL-1β) production (step 2). Although excessive mature secreted IL-18 is detrimental to epithelial integrity, the presence of IL-18 is necessary for IL-22 release by type 3 innate lymphoid cells (ILC3s) (step 3). ILC3-derived IL-22 promotes mucosal barrier protection by inducing mucin synthesis and goblet cell function (step 4). In goblet cells, ROS triggers the NLRP6 inflammasome to elicit mucin granule secretion (step 5). Sentinel goblet cells responding to microbial triggers can signal to adjacent goblet cells to degranulate via gap junctions (step 6). A combination of autophagic proteins, endosomes and NOX-derived ROS are necessary for mucin granule formation in goblet cells (step 7). Both autophagy and mitophagy are induced by hypoxia (step 8). Mitophagy might decrease NLRP3 inflammasome activity, reducing processing of IL-1β and IL-18 (step 9). Inhibition of prolyl hydroxylase enzymes (PHD) by ROS or hypoxia stabilizes hypoxia-inducible factor-1α (HIF1α), regulating barrier protective genes (step 10). Unimpeded or excessive ROS generation during active inflammation can lead to abundant maturation of IL-1β or IL-18 or even inflammasome-mediated cell death (necroptosis and pyroptosis) (step 11). Inflammasome-activation of infiltrating CC-chemokine receptor 2 $(CCR2)^+$ monocytes contributes to active IL-1β (step 12). Mucosal

IL-1β may lead to accumulation of IL-17A-secreting immune cells, such as T helper 17 cells (T_H17) (step 13).

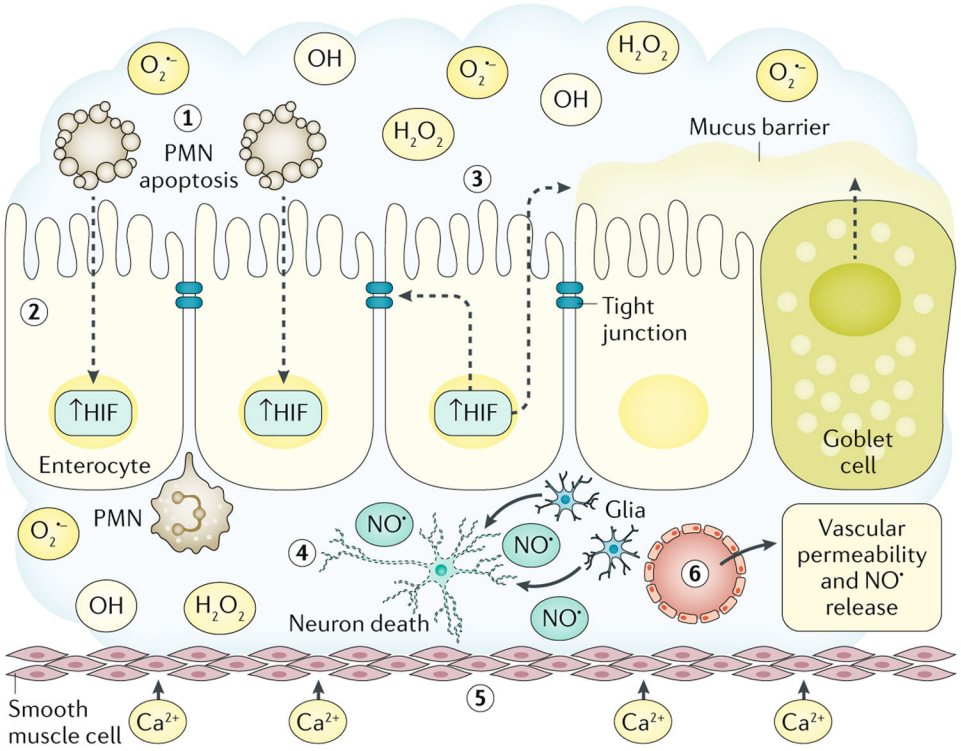

Fig. 3 | ROS collateral damage and gastrointestinal disease.

During active inflammation, reactive oxygen species (ROS; $O_2^{\cdot-}$, $OH^{\cdot}$ and H_2O_2) and reactive nitrogen species generated in the local microenvironment cause collateral tissue damage. Activated, transmigrating polymorphonuclear leukocytes (PMNs) consume large amounts of O_2 in the generation of ROS in the local milieu (step 1). Under these conditions, PMN ROS generation is limited by rapid induction of PMN apoptosis. Such O_2 consumption results in localized hypoxia and the stabilization of epithelial hypoxia-inducible factor (HIF) (step 2). Epithelial HIF stabilization activates a cascade of gene transcription that promotes expression of barrier protective function genes (for example, *TFF3, ABCB1* and *CLDN1*) and mucins in goblet cells (step 3). Within the lamina propria, activation of glial cell inducible nitric oxide synthase and the generation of nitric oxide ($NO^{\cdot}$) leads to enteric nerve cell death, resulting in intestinal dysmotility (step 4). Smooth muscle responses to oxidative stress include increased Ca^{2+} permeability that perpetuates intestinal dysmotility (step 5). An early event in acute mucosal inflammation within the gastrointestinal tract is increased vascular permeability through the generation of $NO^{\cdot}$ by multiple cell types, such as smooth muscle cells, endothelial cells and enteric glia (step 6).