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The authors declare no competing interests.

Abstract

Redox signaling in the colonic 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 and can be detrimental, and thus, maintenance and restitution of a breached intestinal mucosal barrier are paramount to host survival. Redox reactions and redox signaling 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 sub-toxic 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 signaling 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 signaling 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.

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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.

[H1] Introduction

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 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 (AMP) secretion into the lumen of the gut [2,3]. These secretions provide an essential carbon source for the microbial niche, in terms 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 (PMN) and monocytes[7]. A particularly prominent phenotype of acute inflammatory lesions within the intestine is localized accumulation of PMN, termed crypt abscesses. Given the

large amounts of reactive oxygen species (ROS) that can be generated by activated PMN, the crypt abscess represents a major signaling node for reduction-oxidation (redox) signaling[8]. Resident immune cells in the intestine, which include intra-epithelial 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 promoting a regulatory immune response [9-11]. Most of these cell types – immune, epithelia and microorganism — are capable of eliciting and/or circumventing redox signaling with profound implications for mucosal homeostasis.

A significant result of active inflammation in the intestinal mucosae is the localized conversion of molecular O_2 to ROS and resultant hypoxia. At the tissue and cellular level, hypoxia induces an array of genes pivotal to adaptation to low O_2 states. As a global regulator of O_2 homeostasis, the αβ heterodimeric transcription factor hypoxia-inducible factor (HIF) facilitates both O_2 delivery and adaptation to hypoxia[12,13]. HIF-1 and HIF-2 (previously called EPAS) are members of the Per-ARNT-Sim (PAS) family of basic helix-loop-helix (bHLH) transcription factors. HIF activation is dependent upon stabilization of an O_2-dependent degradation (ODD) domain of the α subunit and subsequent nuclear translocation to form a functional complex with HIF-1β and cofactors such as CBP and its ortholog p300. When O_2 supply exceeds demand, iron and O_2 dependent hydoxylation of two prolines (Pro564 and Pro 402) within the ODD of HIF-1 or HIF-2α initiates the association with the von Hippel-Lindau tumor suppressor protein (pVHL) and degradation via ubiquitin-E3 ligase proteasomal targeting [14,15]. A second hypoxic switch operates in the carboxy terminal transactivation domain of HIF-1 or HIF-2α Here, hypoxia blocks the hydroxylation of asparagine-803 so facilitating the recruitment of CBP/p300 [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 comprising 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 microbes is one benefit to the host. In addition to aiding in digestion, microbes benefit the host through the local synthesis of short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate. 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 production of

SCFA-producing microbial species has been associated with colonic disease, including inflammatory bowel disease (IBD)[21-23]. The low-O_2 (anaerobic) conditions that enable SCFA production place unusual metabolic demands on the colonic epithelium[24] and are enhanced during inflammation[25] It is particularly notable that the microbiota is a key regulator of redox potential in the mucosa[26].

Aside from homeostatic and regulatory functions, ROS are well characterized to be produced and contribute to disease processes – acutely during ischemic damage, tissue injury and repair and chronically in inflammatory conditions such as ulcerative colitis, Crohn's disease and colorectal associated cancer. This Review will 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 signaling in microbial-host crosstalk have towards maintaining homeostasis or facilitating disease processes within the gastrointestinal tract.

[H1] Redox signaling in the gut

[H2] Reactive oxygen species generation

Reactive oxygen species (ROS) constitute a major group of potent antimicrobial mediators and redox signaling factors. Both the gastrointestinal mucosa and associated immune cells represent 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 [27]. The reduction and oxidation (redox) state of the gastrointestinal tract is contingent on the balance of antioxidants (for example, heme 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[28]. In the gastrointestinal tract, a variety of reactive oxygen radicals including superoxide ($O_2^{\cdot-}$) and hydroxyl ($^{\cdot}OH$) and non-radicals including hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2) are generated epithelia, endothelia and innate immune cells to implement mucosal defense [29] (Figure 1). Tissue homeostasis is influenced in a variety of ways by the redox state of the tissue, including modulation of signal-transduction pathways (e.g. HIF, NF-kB, Nrf2) [30] 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 defense and cytokine secretion[31]. Of particular significance is the redox state of the ubiquitously-expressed human beta defensin-1 (hBD-1). In the oxidized state, hBD-1 exhibits a limited antimicrobial activity; however, following reduction of the disulfide bridges, hBD-1 alters conformation and displays an enhanced antimicrobial efficacy [32]. Indeed findings from the Wehkamp group demonstrate that the reduced form of hBD-1 is capable of forming net-like structures around bacteria to limit bacteria invasion [33].

[H2] Reactive nitrogen species

Nitric oxide (NO) is a short-lived, lipophilic and freely diffusible signaling molecule synthesized by mammalian cells with a broad spectrum of activities including regulation of blood flow, immune reactions and smooth muscle contraction [34]. NO is generated by the nitric oxide synthase (NOS) enzymes, which convert L-arginine to L-citrulline, liberating NO as a by-product [35]. In the gastrointestinal tract, NO functions as an inhibitory nonadrenergic noncholinerigic neurotransmitter and smooth muscle cell relaxant via activation of guanylate cyclase [36,37]. To date, three isoforms of NOS have been cloned: neuronal NOS (nNOS; NOS1), endothelial NOS (eNOS; NOS3) and inducible NOS (iNOS; NOS2) [38-40]. Interaction of $O_2^{\cdot-}$ with nitric oxide (NO) leads to the formation of peroxynitrite ($ONOO^{-}$) [41]. 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) [41]. $ONOO^{-}$ together with RNS are in turn responsible for nitrosylation of protein tyrosine residues, mitochondrial energy depletion, lipid peroxidation and induction of DNA strand breaks [42]. Nitrosative and oxidative stress have been implicated in a plethora of disease states, including conditions that affect the gastrointestinal tract (namely ischemia reperfusion injury and inflammatory bowel diseases) [30].

[H2] Sources of reactive oxygen species

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 [43], enzymes within the mucosal epithelia and submucosal lamina propria fibroblasts and myofibroblasts such as NADPH oxidases, xanthine oxidase and immune-expressed cyclooxygenases, lipoxygenases and myeloperoxidase[28]. Exogenous or environmental sources of ROS can also trigger oxidative stress, such as ionizing and nonionizing radiation,

chemotherapeutics, xenobiotics, heavy metals and drugs [44,45]. Generation of ROS by cancer chemotherapeutic agents is a major contributor to the toxic side effects associated with these compounds [45]. Cigarette smoke comprises >7,000 chemical compounds and oxidative agents, containing $>10^{14}$ free radicals per inhalation [46]. Tobacco use is known to modulate gastrointestinal diseases and active smokers display an increased risk for colorectal cancer [47] and increased severity of Crohn's disease [48]. For reasons that are not completely clear, tobacco smoke appears to confer a somewhat protective influence to patients with ulcerative colitis [49].

[H1] Mitochondrial metabolism and ROS

While mitochondrial ROS (mtROS) are renowned for causing cellular damage (for example, during ischemia-reperfusion injury [50]), mtROS are now thought to contribute to healthy cellular function in terms of oxygen sensing, as well as disease [51]. Physiological production of mtROS occurs during oxidative phosphorylation and generation of high-energy adenosine triphosphate (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 [52]. Mitochondrial ETC complexes are capable of generating ROS at various sites. Complex I and II release $O_2^{\cdot-}$ into the mitochondrial matrix [53], whereas manganese superoxide dismutase 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, due to a large transmembrane electrical gradient [54]. If $O_2^{\cdot-}$ generated by the mitochondrial ETC is not efficiently converted to H_2O_2, nitric oxide radicals produce peroxynitrite ($ONOO^-$), leading to subsequent irreversible nitration of proteins and enzyme inactivation[42].

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]. There is interest in autophagy as a substantial contributor to intestinal disease mechanisms, especially IBD[55]. 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 micrometer in diameter termed the autophagosome that is subsequently fused with lysosomes for hydrolase-mediated digestion[56]. Considerable overlap exists between cellular stimuli for selective autophagy of damaged organelles (self) and invading microorganisms (non-self)[57]. Mitophagy is a particular type of autophagy, whereby mitochondria are specifically targeted for autophagic lysosomal degradation [58]. Mitophagy is a highly regulated event and some studies indicate

that the mitochondrial 18 kDa translocator protein (TSPO) is central to both regulation of mitochondrial ROS generation and the induction of mitophagy[59]. Interestingly, the overexpression of TSPO in animal models of IBD have revealed that TSPO localizes with epithelial mitochondria [60]. Considering the Endosymbiotic Theory, which postulates the ancient common origin between mitochondria and proteobacteria [61], 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.

[H2] H_2O_2 as a signaling molecule

Oxygen radicals have a limited range of effect due to their short-lived and highly reactive nature[62] Specialized enzymes, such as superoxide dismutases, convert oxygen radicals to the more stable and readily diffusible H_2O_2 (Figure 1). Due to its reduced reactivity, increased half-life and ability to induce reversible protein modification, H_2O_2 can act as a signaling molecule in its own right [62]. H_2O_2 has been demonstrated to oxidize cysteinyl thiol, induce disulphide bond formation and mediate glutathionylation of cysteine or sulphoxidation of methionine residues in numerous proteins. Such modifications can alter protein activity (increased or decreased) but also represents an important antioxidant defense mechanism [63] . In this Review we shall focus primarily on the role of H_2O_2 in mucosal-microbiota crosstalk, but it is noteworthy that other redox signaling mechanisms (e.g. nitrosylation), provide important signaling cues during host-bacterial interactions[64].

[H2] Antioxidant pathways

Regulators of the antioxidant response include enzymes that catalyze and neutralize ROS, ensuring their potent activity is short-lived to minimize collateral damage to the host tissue. Within the gastrointestinal mucosa antioxidant defense systems, superoxide dismutases and glutathione peroxidase enzymes act as detoxification pathways for ROS. Superoxide dismutases (SOD) are metal ion cofactor-requiring enzymes that catalyze the dismutation (i.e. partitioning) of superoxide anions to H_2O_2 and oxygen [65]. In humans there are three SOD isoforms: mitochondrial SOD (manganese-requiring), cytosolic 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 [66]. Indeed increased

SOD activity is associated with mucosal healing of human gastric ulcers, whereas reduced SOD correlates with increased ulcer severity [67].

Conversion of glutathione into oxidized glutathione is performed by the glutathione peroxidase (GPX) enzyme system. In the process, H_2O_2 is enzymatically reduced to H_2O [68]. Within the human gastrointestinal tract, expression of GPX1 is ubiquitous but GPX2 is expressed specifically in epithelial cells [69] and is postulated to protect the mucosa from transporting luminal-derived lipid hydroperoxides [70]. Deletion of either *Gpx1* or *Gpx2* in mice had no phenotypic effect, but double-knockout mice develop spontaneous colitis [71]. Dismutation of H_2O_2 can also be achieved by the enzyme catalase, which converts to $2H_2O_2$ to $2H_2O$ and O_2 [72]. Peroxiredoxins represent another important family of thiol-specific antioxidant enzymes, designated PRDX1-6 and encoded by 6 different genes (reviewed extensively elsewhere [73]). It is notable that there little redundancy exists within this family of proteins, where the loss of individual peroxiredoxin lead to numerous pathologies, including haematological disorders, tumors and increased susceptibility to diseases associated with oxidative stress [74]. Somewhat surprisingly, mice deficient in PRDX2 and PRDX6 are protected from acute colitis [75,76]. While not completely clear, the mechanism of PRDX2-mediated protection may involve ROS-dependent stability of FoxO1 and Foxp3 regulatory T cell development.

A crucial regulator of the antioxidant response is the NF-E2 related factor 2 (Nrf2) transcription factor. 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 (Keap1), an adaptor subunit of Cullin 3 ubiquitin ligase, regulates the function of Nrf2 by acting as a redox sensor (reviewed [77]). Thus, antioxidant pathways provide an equally important and significant balance to redox signaling responses in the gastrointestinal tract.

[H1] Redox signaling in the immune system

Active mucosal inflammation can rapidly deplete both nutrients and oxygen in the immediate environment. For example, when activated, PMN can increase their O_2 demand by as much as 50-fold in the generation of ROS (the so called respiratory burst mediated by NADPH oxidase) necessary to kill microbes following phagocytosis [78]. By contrast, proliferating T cells only moderately increase oxygen consumption during immune responses [79]. 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.

[H2] NADPH oxidases and ROS

The plasma-membrane NADPH oxidase (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 catalyze the conversion of molecular oxygen to superoxide anion[80]. The spectrum of NOX-mediated activity ranges from potent bactericidal capacity of professional phagocytes to critical intracellular signaling 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 permit generation of ROS, including NOX1, NOX3, NOX4, NOX5, DUOX1 and DUOX2 [81] (Figure 1). While DUOX2 and NOX4 are expressed throughout the human gastrointestinal tract, NOX1 expression is highest in the distal colon where it is restricted to the cytosol, presumably to transduce intracellular signaling [29]. By comparison, DUOX2 is expressed on the apical surface of epithelia, ostensibly enabling luminal secretion of ROS [82]. Others have examined the influence of NOX1or DUOX2-derived ROS on *Campylobacter jejuni* infection and discovered that ROS impaired bacterial capsule formation and virulence by altering *C. jejuni* gene expression [83].

[H2] ROS and innate immunity

Innate immune cells, including neutrophils, macrophages and dendritic cells represent the front-line of immune surveillance and defense and generation of ROS is a crucial microbicidal mechanism used by these cells. Activation of the NADPH oxidase complex in innate immune cells elicits a rapid and potent respiratory burst [84]. Defects in phagocyte NADPH oxidase function, such as in patients with chronic granulomatous disease (CGD), lead to leukocytes capable of phagocytosing but with impaired bacterial clearance [85]. The hallmark of CGD is recurrent bacterial and fungal infections. Typically ~40% of patients with CGD develop IBD-like symptoms [86].

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[87] (Figure 2). Typically, TNFα 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 display mixed phenotypes [88]. 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, are an important source of RNS[89]. M2 macrophages are thought to demonstrate a range of activities ranging from wound healing (release of TGF-β) to suppressing T-cell function [11]. Expression of the enzyme Arginase 1 by M2 macrophages depletes L-arginine, resulting in a down-regulation of the T cell receptor (TCR) ζ chain [90], impairing T lymphocyte function and resulting in immunosuppression. Aside from suppressing T cell function, ROS also contribute to regulatory T cell polarization and function[91,92]. The exact molecular mechanisms of how RSO influence regulatory T cell function 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.

[H1] Host–microbial interactions and ROS

The mammalian large intestine plays 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 [93]. 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 signaling to such interactions has emerged as a critical interface to host–microbe interactions in the gut.

[H2] ROS and pathogen niche expansion

Similar to resident gut microorganisms, opportunistic pathogens also use redox reactions to subvert host defenses and establish a niche. One of the most studied in this regard is the invasive enteric pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). This pathogen is associated with acute gastrointestinal inflammation and diarrhea, and elicits neutrophil chemotaxis into the mucosa[94]. Invasion is achieved through two type III secretion

systems that facilitate *S.* Typhimurium to enter and persist inside intestinal epithelial cells and mucosal macrophages[94]. Prior to invasion, *S.* Typhimurium must out-compete the resident gut microbiota. Some studies indicate that inflammation amplifies proliferation of luminal *S.* Typhimurium, enabling it to overgrow other microorganisms[95]. In one report, the Bäumler group demonstrated that inflammation-induced intestinal ROS reacted with luminal thiosulfate to form a new respiratory electron acceptor, tetrathionate [96]. Moreover, *S.* Typhimurium express genes to enable utilization of tetrathionate as an electron acceptor that permits the pathogen to use respiration to outcompete fermenting microorganisms and establish a niche [96]. The authors subsequently demonstrated that this tetrathionate-enabled respiration provided another growth advantage to *S.* Typhimurium, permitting the utilization of epithelial-derived ethanolamine under anaerobic conditions [97].

[H2] 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 [98] and a murine allergic airway model [99]. Other findings suggest 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 [100]. Another study examined the influence of NOX1 or DUOX2-derived ROS on *Campylobacter jejuni* infection and discovered that ROS impaired bacterial capsule formation and virulence by altering *C. jejuni* gene expression [83]. 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 superoxide and H_2O_2 production [101]. An unexpected but intriguing finding from this study was that ablation of epithelial-derived ROS, using an epithelial-restricted Cyba-deficient mouse (absence of the obligatory NOX dimerization partner), led to protection from *C. rodentium*-induced colitis. The authors attribute their findings to an altered gut microbiota with an expansion in H_2O_2-producing lactobacilli, which exert an antimicrobial effect by release of urease, lactic acid and H_2O_2 [102]. Pircalabioru *et al.* demonstrated through the use of catalase to degrade H_2O_2 derived from lactobacilli was responsible for attenuating *C. rodentium* virulence factors [101]. Other findings to support H_2O_2 exerting an antimicrobial function include disruption of microbial intracellular signaling, which affects antioxidant defense and polysaccharide biosynthesis [103]. In the human body, L-amino acids are essential for protein synthesis; however, D-amino acids function in necessary non-ribosome-based roles [104]. Bacteria synthesize and

secrete distinct D-amino acids into the lumen of the colon [105] and the Waldor group demonstrated that microbiota-derived D-amino acids upregulate expression of the host epithelial-expressed enzyme, D-amino acid oxidase (DAO), which is secreted into lumen. Oxidative deamination of D-amino acids by DAO yields H_2O_2 as a by-product and protects from *Vibrio cholerae* pathogenicity [106].

[H2] 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[107] (Figure 1). In both *Drosophila melanogaster* and mouse models, Lactobacilli species were shown to induce epithelial-derived ROS via NOX1 activity, which stimulated epithelial proliferation [108]. Subsequent studies by this group demonstrated dependence on the redox-sensitive transcription factor NRF2 through mechanisms that involve cytoprotection and decreased epithelial apoptosis [109]. 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 (fMLF), in intestinal epithelial wound healing[110,111]. Further studies have revealed that this wound healing response occurs through oxidative inactivation of the regulatory phosphatases PTEN and PTP-PEST, with associated activation of focal adhesion kinase (FAK) and paxillin[112]. Central to such mucosal wound healing responses appears to be the regulation of epithelial cell migration. For example, redox sensitive tyrosine phosphatases (for example, SHP2 and LMW-PTP) that are expressed at the edge of wounded epithelial monolayers are critical to the organization of focal adhesions that organize epithelial migration and wound closure [113]. Loss and gain of function studies have shown that both ANXA1 and NOX1-null mice show substantial deficits in mucosal wound healing responses and that ANXA1 delivery promotes would healing [112]. These investigators have also demonstrated that FPR/NOX2-mediated ROS generation at local intestinal tissue sites select for mucus-resident microorganisms, including *Akkermansia muciniphilia,* that accelerate epithelial wound-healing, in an intestinal epithelial NOX1-dependent fashion [114]

[H1] Consequences of redox signaling

Redox-sensitive signaling pathways are often limited by the availability of extracellular and intracellular oxygen [115]Despite this understanding, ROS generation can occur at surprisingly low oxygen tensions. The neutrophil

NADPH oxidase, for example, is fully functional at ambient oxygen concentrations as low as 1% [78]. Such observations highlight the importance of discriminating oxygen and ROS diffusion within the tissue microenvironment, as well as the variability of oxygen availability within individual tissues. These differences often determine endpoint tissue function and define the adaptability of tissues to hypoxic stress [116].

[H2] 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 [117]. The lumen of the colon is virtually anoxic, mainly due to the microbial biomass[118], and colonic epithelia adjacent to the lumen experience and withstand a pO_2 <10 mmHg[119]. Thus, it might be surprising to discover that epithelial stem cells at the crypt base are highly oxygenated (experiencing a partial pressure of ~100mmHg) [93]. Such differences are compounded by epithelial metabolism and the arrangement of the microvasculature network into counter-current blood flow dynamics within each villous structure [93]. Epithelia adjacent to the lumen effectively exist in a state of 'physiological hypoxia' [120] and are uniquely susceptible to changes in redox state. Experimental use of oxygen-sensitive nitroimidazole compounds has enabled visualization of hypoxia in these tissues both basally and during inflammation [121]. It is notable that these dyes are neither dependent on redox enzymes nor changed by the NADH and NADPH levels [122]. This technology, coupled with immunostaining, has been used to visually reflect the oxygenation of larger solid tumors in mice [123] and might even be a more reliable marker than staining for HIF-1, as it is retained in chronically hypoxic cells [124] Such physiological hypoxia (Figure 1) is reversible by oxygenation of the colonic lumen (for example, using oxygenated perfluorodecalin) [125].

Various factors aside from 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- and glycolysis-related genes (e.g. VEGF, iNOS, GLUT1, PGK1). Less well characterized, but rapidly increasing in number, are genes with associated mucosal-protective functions that enable colonic epithelial cells to restore impaired barrier function (e.g. ITF, MUC3, CLDN1, MDR1) [119]. 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 [121]. 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 [121,128-132] 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[119]. Each of these components are direct transcriptional targets of HIF and contribute fundamentally to the 3D intestinal tissue architecture that forms an intact barrier during homeostasis.

[H2] Induction of epithelial HIF

Following recruitment of immune cells to the mucosa, for instance following induction of experimental colitis, hypoxia extends deeper into tissue [121], a phenomenon termed inflammatory hypoxia [136] (Figure 1). One 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 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 with transmigrated neutrophils within the lumen of the crypt – revealed induction of HIF (monitored by increases in the HIF-target gene *GLUT1)*. However, it is unclear from these studies if HIF stabilization is due to depletion of oxygen or generation of superoxide or H_2O_2, as all are capable of inhibiting the PHD enzymes [25,137].

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 [138,139]. Presumably, these factors function to chelate iron bound within the active site of the PHD enzymes, though this has not been shown conclusively. Fermenting microbiota have also been shown to stabilize HIF in the colon, via short chain fatty acid release, particularly via butyrate production [140]. Butyrate is used as the preferred enterocyte fuel source, oxidizing butyrate to CO_2 [141]. The net effect is epithelial hypoxia due to increased oxygen consumption, likely resulting in PHD enzyme inhibition to facilitate HIF

stabilization [140]. 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 [142].

[H2] Inflammasome activation

The NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) inflammasome is an intracellular multiprotein complex involved in perceived cellular danger response [143]. Pathogen-associated molecular patterns (PAMPs) and host-derived danger-associated molecular patterns (DAMPs) can trigger inflammasome activation [144]. 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 [145]. The role of IL-1β is widely studied in autoimmune diseases; however, in gastrointestinal inflammation its involvement is not fully understood. Studies using chronic mouse models of colitis have demonstrated a role of IL-1β in accumulation of IL-17A secreting CD4+ T helper type 17 cells [146]. One study in 2017 by Neudecker et al., implicated $CCR2^{+}$ monocytes and NLRP3 activity leading to IL-1β production in the pathogenesis of acute colitis in mice[147]. Surprisingly, mice lacking NLRP3 are hypersensitive to experimental colitis, displaying exacerbated immune infiltration and epithelial damage, primarily due to a loss of IL-18 [148]. 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 [149]. Conversely, activation of ROS, ostensibly by NADPH oxidases [150] has been shown to stimulate the NLRP3 inflammasome. However, patients with CGD with NADPH-deficient macrophages display normal inflammasome activation in several studies [151,152], implicating other stimuli aside from NADPH-oxidase-derived ROS. Another abundant source of cellular ROS are mitochondria, which release ROS in response to elevated metabolism, hypoxia or membrane damage [153]. 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 [154]. 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 [154].

[H2] Goblet cell mucus secretion

Goblet cells are specialized epithelial cells that protect the barrier from microbial invasion by secretion of a mucus hydrogel [155]. 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 centered on understanding the regulation of mucin packaging and secretion at baseline and in response to microorgansims detection [156], which has led to the suggestion that goblet cells actually represent an unappreciated and distinct innate immune cell [4,157]. Another secretory epithelial lineage, Paneth cells — which are tasked with antimicrobial peptide secretion and defense of the intestinal stem cell niche — rely on autophagy to organize secretory granules [158]. By contrast, autophagy compromised goblet cells displayed normal mucin packaging into granules [159]. However, a combination of autophagy and NADPH oxidase-derived ROS were found to be essential for mucin release by goblet cells (Figure 2). Patel et al. demonstrated that amphisome-like vesicles form in goblet cells following autophagosome and endosome fusion and these specialized organelles regulate mucin secretion [159]. It was subsequently demonstrated that the NLRP6 inflammasome is crucial for promoting goblet cell mucin release in response to proximity with microorganisms [160]. In 2016, the Hansson group proposed the existence of a 'sentinel' goblet cell [161] based on proximity to the crypt entrance. This sentinel goblet cell non-specifically endocytoses and responds to TLR ligands, stimulating NOX1or 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 neighboring goblet cells [161].

Secretion of additional mucins in response to detection of microbial proximity is obviously one approach to repel a microbial onslaught, but goblet cell hyperplasia represents an alternative mechanism. As mucus erosion and goblet cell depletion are pathological hallmarks of ulcerative colitis[162], repletion of both goblet cells and their mucin granule contents is necessary for epithelial barrier restitution (Figure 3). As mentioned previously, IL-18 secretion is stimulated by ROS-mediated inflammasome activation[145], where some findings reveal distinct and opposing roles for IL-18 and IL-22 signaling in regulating goblet cell homeostasis. For example, Flavell and colleagues demonstrated using various intestinal epithelial-specific knockout mice to target IL-18 signaling, that excess IL-18 promotes goblet cell depletion and colitis. Moreover, IL-18 appears to suppress goblet cell differentiation markers

[163]. Contrastingly, immune cell-derived IL-22 has well-recognized protective mucosal effects via promoting stem cell differentiation, mucin synthesis (mucin 2), antimicrobial peptide (RegIIIγ) and goblet cell function (Fut 2 expression) [164-166]. The recently characterized type-3 innate lymphoid cells (ILC3) are the major source of IL-22 within the intestinal mucosa [167]. In fact, during *Toxoplasma gondii* infection, ILCs and T cells required epithelial-derived inflammasome-processed IL-18 in order to release IL-22 [168]. Thus, a combination of redox signaling, inflammasome activity and immune crosstalk might hold the key to homeostasis between IL-18 and IL-22 signaling and indeed mucosal-microbiota homeostasis. Moreover, IL-1β can both induce activation of ILC3 and contribute to plasticity (in concert with other factors, including retinoic acid) and reprograming of ILC1 and ex-ILC3 to ILC3s [169].

[H1] Redox signaling in intestinal disorders

[H2] Ischemia–reperfusion injury

Ischemia is defined as insufficient blood flow to tissues, resulting in disruption of cell function and ultimately necrosis. A variety of tissue insults can lead to intestinal ischemia, including trauma, stroke and atherosclerosis, and reperfusion (restitution of blood supply) following ischemia can result in aggravated tissue damage. The intestine is particularly sensitive to ischemia–reperfusion injury (I/RI) [170]. Ischemia 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[171]. Constitutively expressed COX1 and the inducible isoform COX2 are responsible for catalyzing the conversion of arachidonic acid to prostaglandins [172]. The primary prostaglandin studied in this context is prostaglandin E_2 (PGE_2) [173], 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 [174]. Infiltrating leukocytes expressing 5-lipoxygenase and 15-lipoxygenase leukotriene B_4 (LTB_4), produced by endothelial cells, a potent chemoattractant of neutrophils and facilitates neutrophil adhesion and degranulation [175]. Following reperfusion, a necessary substrate (oxygen) becomes available to enable the *de novo* synthesis of an 'eicosanoid storm', where is essence, bioactive lipids of the eicosanoid family become significantly amplified in their production [176]. This combination of lipid-mediator and neutrophil accumulation in the tissue milieu results in

another consequence upon I/RI, namely lipid peroxidation[177], the oxidative degradation of lipids that result in plasma membrane and organelle damage. ROS generated by reoxygenated neutrophils have long been recognized as instigators of lipid peroxidation in intestinal I/RI, resulting in barrier disruption[50,135,170,177]. Indeed, treatment with superoxide dismutase in a murine intestinal I/RI 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 I/RI mostly hinge on reducing neutrophil recruitment signals and leukocyte activation [178]. The anti-inflammatory influences of carbon monoxide, derived from endogenous heme oxygenase, might be a promising therapeutic strategy to attenuate damage from I/RI [179]. Multiple lines of evidence have revealed that the activation of heme oxygenase effectively promotes cytoprotection and inhibits the pro-inflammatory signatures elicited from multiple cell types during intestinal I/RI[179]. Strategies to induce heme oxygenase and carbon monoxide release are in development and include HO1 fusion proteins, small molecule heme oxygenase inducers, bilirubin, glutamine and inhaled carbon monoxide[179].

[H2] Ischemic preconditioning

Aside from leukocyte-derived sources of ROS, the mucosa itself can contribute significantly to redox-mediated damage during intestinal ischemia. High concentrations of ATP are released extracellularly during ischemia, which are ultimately catabolized to hypoxanthine [180]. Concomitantly, ischemic stress results in the conversion of xanthine dehydrogenase to xanthine oxide [181]. Following tissue reperfusion, the combination of hypoxanthine, xanthine oxide and newly available molecular oxygen yields additional sources of tissue superoxide anion [182]. Although some limited therapeutic success has arisen from scavenging ROS or targeting inflammatory mediators, one of the more promising strategies to reduce I/RI is ischemic preconditioning (or hypoxic preconditioning), whereby cells or tissues are exposed to brief and intermittent periods of non-lethal ischemia. Such treatments have been shown to protect organs that experience a major ischemic 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 HO1[184]. Khoury et al. identified extracellular adenosine released by hypoxic preconditioned intestinal epithelia as the major anti-inflammatory factor responsible for hypoxic preconditioning [185]. This protective role of adenosine in ischemic preconditioning corresponded with the inhibition of NF-kappaB via deneddylation (where NEDD8 is removed from

a conjugated protein) of cullin-1 (Cul1), a component of the proteasomal degradation pathway important in the activation of NF-kappaB[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 adenosine monophosphate analog that functionally inhibits Nedd8 activating enzyme and results in the de-neddylation of Cul1 and Cul2[187,188] and has proven to be a potent HIF stabilizer in cultured cells[189]. In this regard, HIF might function to promote tissue ischemic preconditioning, which has been shown in some studies[13], and small molecule stabilizers of HIF (esp. PHD inhibitors) show promise in protection from damage associated with I/RI[178].

Extracellular adenosine is derived from the enzymatic degradation of ATP via the action of surface apyrases (e.g. CD39) and ecto-5'-nucleotidase (CD73) [190]. 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 (ENT)1 and ENT2, resulting in reduced uptake of extracellular adenosine providing more available for extracellular signaling [191]. Extracellular adenosine signals through activation of any of four surface G-protein coupled receptors. Activation of the A1AR or the A3AR results in decreased intracellular cAMP levels (Gαi-coupled), whereas adenosine binding to the high affinity A2AAR or the low affinity A2BAR is associated with elevation of cAMP (Gαs-coupled) [192]. The predominant receptor-mediated signaling associated with intestinal epithelial cells is A2BAR and the crystal structure of agonist and antagonist bound A2AAR has been resolved [193]. 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 [194,195]. These studies have shown important protective roles for A2BAR in experimental colitis [194] and intestinal I/RI [196,197] that results in diminished inflammation [198].

A number of sources of nucleotides exist in inflamed and ischemic tissue. Many cell types actively release nucleotides, particularly in the form of ATP or ADP [192]. Programmed cell death (apoptosis) is associated with the generation of large amounts of ATP during ischemia 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 [199]. Other studies have shown that inflammatory cells, such as neutrophils, can release ATP in an active manner though connexin 43 hemichannels [200,201]. Platelets release nucleotides at high concentration upon activation and are

also an important source of extracellular nucleotides during ischemia. 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[202]. As originally described by Madara et al [203], 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.

[H2] 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 [55]. 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 that susceptibility to IBD is dictated by a combination of genetic, environmental and microbial risk factors [55]. 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 signaling and in bystander damage to surrounding tissue[30].

Does excessive ROS or insufficient ROS contribute to IBD? Evidence exists to support both excessive ROS or 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 etiological 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 superoxide generation in CGD is a major risk factor for IBD[204].

As alluded to earlier, the majority of patients with CGD develop IBD-like symptoms [86]. A potentially confounding issue for research in this field is the mouse models used to address the roles of phagocyte-derived ROS versus mucosal sources. Campbell et al., [8] using a TNBS (2,4,6-trinitrobenzenesulfonic acid) 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 to wild-type control mice. Conversely Bao et al., used the same mice in a dextran sulfate sodium (DSS) model of colitis and found no difference in weight loss or disease severity when compared to wild type controls [205]. 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[206]. Under such circumstances, it could be argued that phagocyte-derived ROS might not be contributing to denudation of colonic epithelia, therefore only genes or therapeutic intervention that influence epithelial viability or turnover will have an appreciable effect. By contrast, TNBS involves pre-sensitizing the host immune system to haptenized microbial antigens, with subsequent colonic exposure to the haptenizing agent[206]. 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 [206] Moreover, immune infiltrates and inflammatory mediators differ substantially between the models [206]As such, DSS represents a wound model, whereas TNBS 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 I/RI. For instance, proinflammatory mediators such as TNF-α and PGE_2 are implicated in both I/RI and Crohn's disease [50,207]. Involvement of neutrophils, monocytes and leukocyte-derived ROS have been implicated in both ulcerative colitis and I/RI in the colon and intestine [50,208]. Similarly, epithelial barrier disruption and enhanced microbial translocation are features of both IBD and I/RI [50,209]. Studies have also suggested that shifts in the gut microbiome (dysbiosis) might contribute to both I/RI and IBD[50,210]. One important caveat to this understanding is the observation that antibody-mediated neutrophil depletion strategies in intestinal I/RI models do not appear to influence injury endpoints [211], whereas neutrophil depletion substantially enhances tissue damage in multiple colitis models [212]. Another common feature between IBD and I/RI models, is accumulation of extracellular ATP in colitis models has been demonstrated to contribute to the inflammatory process, in part via stimulation of the P2X7 receptor [213]. Also similar to ischemic preconditioning is that extracellular adenosine appears to confer mucosal protection during colitis, principally via A2BAR signaling. 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 [194,195].

[H2] 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 [214]. Implications of excessive ROS-mediated tissue damage in the gastrointestinal tract include alterations of absorptive function, barrier dysfunction and dysmotility [215]. Numerous studies have, for example, indicated malabsorption of nutrients in the intestine following I/RI and in IBD[216,217]. Colonic epithelia, by contrast, are tasked predominantly with the reuptake of water from the faecal stream, so disruption to colonic absorption in IBD manifests as diarrhea [218]. Extensive tissue damage from excessive ROS (for example, lipid peroxidation, protein chlorination of mucosal barrier proteins) and immune mediators such as TNF-α and IFN-γ increase mucosal permeability by modulating tight junction function [219] (Figure 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 [220]. It is notable that increases in vascular permeability might precede increases in epithelial permeability during active mucosal inflammation (see Figure 3). Tolstanova, et al used four murine models of colitis to demonstrate that early endothelial damage resulted in perivascular edema and epithelial hypoxia, which contributed to the stabilization of HIF within the mucosa [221]. Evidence for early ROS-mediated endothelial damage were demonstrated at the level of electron microscopy and were consistent in genetic models of colitis, including IL-10-/- and Gα-i2 -/- [221]. Finally, it is likely that gut motility is affected through redox-sensitive mechanisms. For example, Brown, et al showed that enteric neuron death during active colitis was mediated by nitric oxide derived from glial cells[222] (Figure 3). Such neurotoxic activity was driven by nitric oxide influences on connexin-43 activity. Moreover, exposure of sub-mucosal smooth muscle cells to microbial lipopolysaccharide probably contributes to dysmotility through the generation of large amounts of ROS and RNS [223]. Taken together, these studies implicate collateral tissue damage associated with oxidative stress in active intestinal inflammation.

[H1] Conclusions

In conclusion, the gastrointestinal tract represents a particularly austere environment for redox-sensitive signaling. The combination of multiple sources of reactive oxygen or nitrogen species 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 anti-microbial 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, endothelial, fibroblasts) as well as the gut microbiota are coupled with the differing potencies, toxicities and halflives 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 mediate collateral tissue damage and endpoint organ function. A better understanding of the basic molecular signals, transcriptional programs and the environmental clues that regulate mucosal redox state (Box 1) are likely to provide new insight toward the development of novel therapies for diseases such as IBD.

[H1] Box 1: Knowledge gaps and future research directions

- What host metabolic factors control to redox state threshold and under what conditions are they generated?
- Could microbiota-derived factors that influence redox 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 HIF (e.g., via PHD inhibition) a viable therapeutic option for mucosal disease?
- What pharmacological approaches best mimic ischemic preconditioning and under what circumstances might this approach benefit the host?
- For therapeutic targeting of redox pathways, how might we maximize the beneficial influence of redox signaling and minimize bystander tissue damage?

Figure 1: Host-microbial redox signaling during hypoxia.

Enzymatic utilization of molecular oxygen within the intestinal mucosa facilitates redox signaling and results in both spatial and dynamic patterns of oxygen availability. a) In the healthy intestinal mucosa, a steep oxygen 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 (~100mm Hg) compared with the luminal-effacing epithelia (<10mm Hg) 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 the HIF transcription factor. Gut microbiota secreting short chain fatty acids (SCFA), particularly butyrate, contribute to this physiological hypoxia through increased oxidative phosphorylation. Luminal redox signaling initiated by resident microorganisms releasing D-amino acids (D-aa) stimulates the epithelium to secrete D-amino acid oxidase (DAO) into the lumen, which is subsequently yields H_2O_2. Apical expression of epithelial DUOX2 probably results in luminal secretion of H_2O_2, which contributes to limiting opportunistic pathogen niche expansion. Probiotic Lactobacilli up-regulate epithelial NOX1 expression, which in turn induces DUOX2. Epithelial expressed NOX1 and DUOX2, utilizing microenvironmental oxygen, generate oxygen radicals to further contribute to luminal release of H_2O_2. b) During inflammatory hypoxia, infiltrating PMN and monocytes, expressing NOX2 generate superoxide ($O_2^{\cdot-}$), resulting in inhibition of PHD enzymes and stabilization of HIF deep into the crypt. HIF transcriptional activity induces expression of barrier protective factors – antimicrobial peptides (AMPs), Mucin 3 (Muc3) and intestinal trefoil factor (TFF3). Certain opportunistic pathogens release siderophores, sequestering iron and inhibiting PHD enzymes. Sulfur metabolism of the mucosa can be hijacked by opportunistic pathogens – H_2S is routinely detoxified to thiosulfate ($S_2O_3^{2-}$); 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.

Figure 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 NOX1 or NOX4. **a.** In response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), epithelia recruit and activate the Nox1/4 complex, stimulating superoxide

and hydrogen peroxide generation (sources of ROS). Both enzymatic and mitochondrial-derived ROS can trigger the activity of epithelial inflammasomes. **b.** In colonic epithelia, ROS-stimulated NLRP3 inflammasome assembly leads to IL-18 (and IL-1β) production. Whereas excessive mature secreted IL-18 is detrimental to epithelial integrity, presence of IL-18 is necessary for IL-22 release by type 3 innate lymphoid cells (ILC3s). **c.** ILC3-derived IL-22 promotes mucosal barrier protection by inducing mucin synthesis and goblet cell function. **d.** In goblet cells, ROS triggers the NLRP6 inflammasome to elicit mucin granule secretion. **e.** Sentinel goblet cells responding to microbial triggers can signal to adjacent goblet cells to degranulate via GAP junctions. **f.** A combination of autophagic proteins, endosomes and NADPH oxidase-derived ROS are necessary for mucin granule formation in goblet cells. **g.** Both autophagy and mitophagy are induced by hypoxia. Mitophagy might decrease NLRP3 inflammasome activity, reducing processing of IL-1β and IL-18. **h.** Inhibition of PHD enzymes by ROS or hypoxia stabilizes HIF1α transcription factor, regulating barrier protective genes. **i.** Unimpeded or excessive ROS generation can lead to abundant maturation of IL-1β or IL-18 or even inflammasome-mediated cell death (necroptosis/pyroptosis). **j.** Inflammasome-activation of CCR2+ infiltrating monocytes contribute to active IL-1β. Mucosal IL-1β may lead to accumulation of IL-17A secreting immune cells.

Figure 3: Reactive oxygen species collateral damage and gastrointestinal disease

During active inflammation, reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated within the local microenvironment affect collateral tissues to cause damage. Activated, transmigrating neutrophils (PMN) consume large amounts of O_2 in the generation of amounts of ROS ($O2^{-\cdot}$, $OH^{\cdot}$ and H_2O_2) in the local milieu. Such O_2 consumption results in localized hypoxia and the stabilization of epithelial HIF. Epithelial HIF stabilization activates a cascade of gene transcription that promotes expression of barrier protective function genes and mucins in goblet cells. Within the lamina propria, activation of glial cell inducible nitric oxide synthase and the generation of nitric oxide ($NO^{\cdot}$) results in enteric nerve cell death to result in dysmotility. Smooth muscle responses to oxidant stress include increased Ca^{2+} permeability that perpetuates intestinal dysmotility. An early event in acute mucosal inflammation within the gastrointestinal tract is increased vascular permeability and the generation of $NO^{\cdot}$ to promote local tissue damage.

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.

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Author contributions

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

Competing interests statement

The authors declare no competing interests.

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References:

1 France, M. M. & Turner, J. R. The mucosal barrier at a glance. *J Cell Sci* **130**, 307-314, doi:10.1242/jcs.193482 (2017).

2 Crosnier, C., Stamataki, D. & Lewis, J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet* **7**, 349-359, doi:10.1038/nrg1840 (2006).

3 Clevers, H. C. & Bevins, C. L. Paneth cells: maestros of the small intestinal crypts. *Annu Rev Physiol* **75**, 289-311, doi:10.1146/annurev-physiol-030212-183744 (2013).

4 Johansson, M. E. & Hansson, G. C. Immunological aspects of intestinal mucus and mucins. *Nat Rev Immunol* **16**, 639-649, doi:10.1038/nri.2016.88 (2016).

5 Tailford, L. E., Crost, E. H., Kavanaugh, D. & Juge, N. Mucin glycan foraging in the human gut microbiome. *Front Genet* **6**, 81, doi:10.3389/fgene.2015.00081 (2015).

6 Taylor, C. T. & Colgan, S. P. Regulation of immunity and inflammation by hypoxia in immunological niches. *Nat Rev Immunol.* **17**, 774-785. (2017).

7 Lewis, J. S., Lee, J. A., Underwood, J. C., Harris, A. L. & Lewis, C. E. Macrophage responses to hypoxia: relevance to disease mechanisms. *J Leukoc Biol* **66**, 889-900 (1999).

8 Campbell, E. L. *et al.* Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. *Immunity.* **40**, 66-77. (2014).

9 Chang, S. Y., Ko, H. J. & Kweon, M. N. Mucosal dendritic cells shape mucosal immunity. *Exp Mol Med* **46**, e84, doi:10.1038/emm.2014.16 (2014).

10 Sun, M., He, C., Cong, Y. & Liu, Z. Regulatory immune cells in regulation of intestinal inflammatory response to microbiota. *Mucosal Immunol* **8**, 969-978, doi:10.1038/mi.2015.49 (2015).

11 Davies, L. C., Jenkins, S. J., Allen, J. E. & Taylor, P. R. Tissue-resident macrophages. *Nat Immunol* **14**, 986-995, doi:10.1038/ni.2705 (2013).

12 Semenza, G. L. Oxygen sensing, homeostasis, and disease. *NEJM* **365**, 537-547 (2011).

13 Semenza, G. L. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu Rev Pathol* **9**, 47-71 (2014).

14 Maxwell, P. H. *et al.* The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271-275 (1999).

15 Tanimoto, K., Makino, Y., Pereira, T. & Poellinger, L. Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. *Embo J* **19**, 4298-4309 (2000).

16 Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J. & Murray, L. W. Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. *Science* **295**, 858-861 (2002).

17 Sender, R., Fuchs, S. & Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol.* **14**, e1002533. (2016).

18 McCracken, V. J. & Lorenz, R. G. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. *Cell Microbiol* **3**, 1-11 (2001).

19 Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220-230, doi:10.1038/nature11550 (2012).

20 Hamer, H. M. *et al.* Review article: the role of butyrate on colonic function. *Alimentary pharmacology & therapeutics* **27**, 104-119, doi:10.1111/j.1365-2036.2007.03562.x (2008).

21 Machiels, K. *et al.* A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut*, doi:10.1136/gutjnl-2013-304833 (2013).

22 Eeckhaut, V. *et al.* Butyricicoccus pullicaecorum in inflammatory bowel disease. *Gut* **62**, 1745-1752, doi:10.1136/gutjnl-2012-303611 (2013).

23 Sokol, H. *et al.* Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflammatory bowel diseases* **15**, 1183-1189, doi:10.1002/ibd.20903 (2009).

24 Tremaroli, V. & Backhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature.* **489**, 242-249. doi: 210.1038/nature11552. (2012).

25 Campbell, E. L. *et al.* Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. *Immunity* **40**, 66-77 (2014).

26 Jones, R. M. & Neish, A. S. Redox signaling mediated by the gut microbiota. *Free Radic Biol Med.* **105**, 41-47. (2017).

27 Halliwell, B. & Gutteridge, J. M. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* **186**, 1-85 (1990).

28 Kulkarni, A. C., Kuppusamy, P. & Parinandi, N. Oxygen, the lead actor in the pathophysiologic drama: enactment of the trinity of normoxia, hypoxia, and hyperoxia in disease and therapy. *Antioxid Redox Signal* **9**, 1717-1730, doi:10.1089/ars.2007.1724 (2007).

29 Bedard, K. & Krause, K. H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* **87**, 245-313, doi:10.1152/physrev.00044.2005 (2007).

30 Biasi, F., Leonarduzzi, G., Oteiza, P. I. & Poli, G. Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets. *Antioxid Redox Signal.* **19**, 1711-1747. (2013).

31 Bals, R. Epithelial antimicrobial peptides in host defense against infection. *Respir Res* **1**, 141-150. (2000).

32 Schroeder, B. O. *et al.* Reduction of disulphide bonds unmasks potent antimicrobial activity of human beta-defensin 1. *Nature* **469**, 419-423, doi:10.1038/nature09674 (2011).

33 Raschig, J. *et al.* Ubiquitously expressed Human Beta Defensin 1 (hBD1) forms bacteria-entrapping nets in a redox dependent mode of action. *PLoS Pathog* **13**, e1006261, doi:10.1371/journal.ppat.1006261 (2017).

34 Palmer, R. M., Ferrige, A. G. & Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**, 524-526, doi:10.1038/327524a0 (1987).

35 Palmer, R. M., Ashton, D. S. & Moncada, S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**, 664-666, doi:10.1038/333664a0 (1988).

36 Stark, M. E., Bauer, A. J., Sarr, M. G. & Szurszewski, J. H. Nitric oxide mediates inhibitory nerve input in human and canine jejunum. *Gastroenterology* **104**, 398-409 (1993).

37 Stark, M. E. & Szurszewski, J. H. Role of nitric oxide in gastrointestinal and hepatic function and disease. *Gastroenterology* **103**, 1928-1949 (1992).

38 Sessa, W. C. *et al.* Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. *J Biol Chem* **267**, 15274-15276 (1992).

39 Xie, Q. W. *et al.* Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* **256**, 225-228 (1992).

40 Bredt, D. S. *et al.* Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* **351**, 714-718, doi:10.1038/351714a0 (1991).

41 Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A. & Freeman, B. A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* **87**, 1620-1624 (1990).

42 Beckman, J. S. & Koppenol, W. H. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* **271**, C1424-1437 (1996).

43 Poyton, R. O., Castello, P. R., Ball, K. A., Woo, D. K. & Pan, N. Mitochondria and hypoxic signaling: a new view. *Ann N Y Acad Sci* **1177**, 48-56, doi:10.1111/j.1749-6632.2009.05046.x (2009).

44 Riley, P. A. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* **65**, 27-33 (1994).

45 Conklin, K. A. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. *Integr Cancer Ther* **3**, 294-300, doi:10.1177/1534735404270335 (2004).

46 Church, D. F. & Pryor, W. A. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect* **64**, 111-126 (1985).

47 Anderson, J. C. *et al.* Smokers as a high-risk group: data from a screening population. *J Clin Gastroenterol* **43**, 747-752, doi:10.1097/MCG.0b013e3181956f33 (2009).

48 van der Heide, F. *et al.* Differences in genetic background between active smokers, passive smokers, and non-smokers with Crohn's disease. *Am J Gastroenterol* **105**, 1165-1172, doi:10.1038/ajg.2009.659 (2010).

49 Loftus, E. V., Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* **126**, 1504-1517 (2004).

50 Kalogeris, T., Baines, C. P., Krenz, M. & Korthuis, R. J. Ischemia/Reperfusion. *Compr Physiol.* **7**, 113-170. (2016).

51 Balaban, R. S., Nemoto, S. & Finkel, T. Mitochondria, oxidants, and aging. *Cell* **120**, 483-495, doi:10.1016/j.cell.2005.02.001 (2005).

52 West, A. P., Shadel, G. S. & Ghosh, S. Mitochondria in innate immune responses. *Nat Rev Immunol* **11**, 389-402, doi:10.1038/nri2975 (2011).

53 Genova, M. L. *et al.* The site of production of superoxide radical in mitochondrial Complex I is not a bound ubisemiquinone but presumably iron-sulfur cluster N2. *FEBS Lett* **505**, 364-368 (2001).

54 Sabharwal, S. S. & Schumacker, P. T. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nat Rev Cancer* **14**, 709-721, doi:10.1038/nrc3803 (2014).

55 McGovern, D. P., Kugathasan, S. & Cho, J. H. Genetics of Inflammatory Bowel Diseases. *Gastroenterology.* **149**, 1163-1176 (2015).

56 Ohsumi, Y. Historical landmarks of autophagy research. *Cell Res.* **24**, 9-23. (2014).

57 Levine, B., Mizushima, N. & Virgin, H. W. Autophagy in immunity and inflammation. *Nature* **469**, 323-335, doi:10.1038/nature09782 (2011).

58 Lemasters, J. J. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res* **8**, 3-5, doi:10.1089/rej.2005.8.3 (2005).

59 Gatliff, J. & Campanella, M. TSPO is a REDOX regulator of cell mitophagy. *Biochemical Society transactions* **43**, 543-552, doi:10.1042/bst20150037 (2015).

60 Ostuni, M. A. *et al.* Overexpression of translocator protein in inflammatory bowel disease: potential diagnostic and treatment value. *Inflammatory bowel diseases* **16**, 1476-1487 (2010).

61 Timmis, J. N., Ayliffe, M. A., Huang, C. Y. & Martin, W. Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat Rev Genet* **5**, 123-135, doi:10.1038/nrg1271 (2004).

62 Stone, J. R. & Yang, S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* **8**, 243-270, doi:10.1089/ars.2006.8.243 (2006).

63 Davies, M. J. Protein oxidation and peroxidation. *Biochem J.* **473**, 805-825. (2016).

64 Vazquez-Torres, A. & Baumler, A. J. Nitrate, nitrite and nitric oxide reductases: from the last universal common ancestor to modern bacterial pathogens. *Curr Opin Microbiol.* **29**, 1-8. (2016).

65 Zelko, I. N., Mariani, T. J. & Folz, R. J. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic Biol Med* **33**, 337-349 (2002).

66 Klinowski, E., Broide, E., Varsano, R., Eshchar, J. & Scapa, E. Superoxide dismutase activity in duodenal ulcer patients. *Eur J Gastroenterol Hepatol* **8**, 1151-1155 (1996).

67 Naito, Y. *et al.* Changes in superoxide dismutase activity in the gastric mucosa of peptic ulcer patients. *J Clin Gastroenterol* **14 Suppl 1**, S131-134 (1992).

68 Toppo, S., Vanin, S., Bosello, V. & Tosatto, S. C. Evolutionary and structural insights into the multifaceted glutathione peroxidase (Gpx) superfamily. *Antioxid Redox Signal* **10**, 1501-1514, doi:10.1089/ars.2008.2057 (2008).

69 Chu, F. F., Doroshow, J. H. & Esworthy, R. S. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J Biol Chem* **268**, 2571-2576 (1993).

70 Wingler, K., Muller, C., Schmehl, K., Florian, S. & Brigelius-Flohe, R. Gastrointestinal glutathione peroxidase prevents transport of lipid hydroperoxides in CaCo-2 cells. *Gastroenterology* **119**, 420-430 (2000).

71 Esworthy, R. S. *et al.* Mice with combined disruption of Gpx1 and Gpx2 genes have colitis. *Am J Physiol Gastrointest Liver Physiol* **281**, G848-855 (2001).

72 Schrader, M. & Fahimi, H. D. Peroxisomes and oxidative stress. *Biochim Biophys Acta* **1763**, 1755-1766, doi:10.1016/j.bbamcr.2006.09.006 (2006).

73 Knoops, B., Argyropoulou, V., Becker, S., Ferte, L. & Kuznetsova, O. Multiple Roles of Peroxiredoxins in Inflammation. *Mol Cells* **39**, 60-64, doi:10.14348/molcells.2016.2341 (2016).

74 Hampton, M. B. & O'Connor, K. M. Peroxiredoxins and the Regulation of Cell Death. *Mol Cells.* **39**, 72-76. (2016).

75 Won, H. Y. *et al.* Ablation of peroxiredoxin II attenuates experimental colitis by increasing FoxO1-induced Foxp3+ regulatory T cells. *J Immunol* **191**, 4029-4037, doi:10.4049/jimmunol.1203247 (2013).

76 Melhem, H. *et al.* Prdx6 Deficiency Ameliorates DSS Colitis: Relevance of Compensatory Antioxidant Mechanisms. *J Crohns Colitis* **11**, 871-884, doi:10.1093/ecco-jcc/jjx016 (2017).

77 Suzuki, T. & Yamamoto, M. Stress-sensing mechanisms and the physiological roles of the Keap1-Nrf2 system during cellular stress. *J Biol Chem* **292**, 16817-16824, doi:10.1074/jbc.R117.800169 (2017).

78 Gabig, T. G., Bearman, S. I. & Babior, B. M. Effects of oxygen tension and pH on the respiratory burst of human neutrophils. *Blood* **53**, 1133-1139 (1979).

79 Fox, C. J., Hammerman, P. S. & Thompson, C. B. Fuel feeds function: energy metabolism and the T-cell response. *Nat Rev Immunol* **5**, 844-852, doi:nri1710 [pii]

10.1038/nri1710 [doi] (2005).

80 Campbell, E. L. & Colgan, S. P. Neutrophils and inflammatory metabolism in antimicrobial functions of the mucosa. *J Leukoc Biol.* **98**, 517-522. doi: 510.1189/jlb.1183MR1114-1556R. Epub 2015 Feb 1124. (2015).

81 Geiszt, M. NADPH oxidases: new kids on the block. *Cardiovascular research* **71**, 289-299, doi:10.1016/j.cardiores.2006.05.004 (2006).

82 Rada, B. & Leto, T. L. Oxidative innate immune defenses by Nox/Duox family NADPH oxidases. *Contributions to microbiology* **15**, 164-187, doi:10.1159/000136357 (2008).

83 Corcionivoschi, N. *et al.* Mucosal reactive oxygen species decrease virulence by disrupting Campylobacter jejuni phosphotyrosine signaling. *Cell host & microbe* **12**, 47-59, doi:10.1016/j.chom.2012.05.018 (2012).

84 McPhail, L. C., Henson, P. M. & Johnston, R. B., Jr. Respiratory burst enzyme in human neutrophils. Evidence for multiple mechanisms of activation. *J Clin Invest.* **67**, 710-716. (1981).

85 Dinauer, M. C., Orkin, S. H., Brown, R., Jesaitis, A. J. & Parkos, C. A. The glycoprotein encoded by the X-linked chronic granulomatous disease locus is a component of the neutrophil cytochrome b complex. *Nature* **327**, 717-720, doi:10.1038/327717a0 (1987).

86 Werlin, S. L., Chusid, M. J., Caya, J. & Oechler, H. W. Colitis in chronic granulomatous disease. *Gastroenterology.* **82**, 328-331. (1982).

87 Mantovani, A., Sica, A. & Locati, M. Macrophage polarization comes of age. *Immunity.* **23**, 344-346. (2005).

88 Hume, D. A. The Many Alternative Faces of Macrophage Activation. *Front Immunol.* **6**, 10.3389/fimmu.2015.00370. (2015).

89 MacMicking, J., Xie, Q. W. & Nathan, C. Nitric oxide and macrophage function. *Annu Rev Immunol* **15**, 323-350, doi:10.1146/annurev.immunol.15.1.323 (1997).

90 Rodriguez, P. C. *et al.* Regulation of T cell receptor CD3zeta chain expression by L-arginine. *J Biol Chem* **277**, 21123-21129, doi:10.1074/jbc.M110675200 (2002).

91 Efimova, O., Szankasi, P. & Kelley, T. W. Ncf1 (p47phox) is essential for direct regulatory T cell mediated suppression of CD4+ effector T cells. *PLoS One* **6**, e16013, doi:10.1371/journal.pone.0016013 (2011).

92 Kraaij, M. D. *et al.* Induction of regulatory T cells by macrophages is dependent on production of reactive oxygen species. *Proc Natl Acad Sci U S A* **107**, 17686-17691, doi:10.1073/pnas.1012016107 (2010).

93 Zheng, L., Kelly, C. J. & Colgan, S. P. Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A Review in the Theme: Cellular Responses to Hypoxia. *Am J Physiol Cell Physiol.* **309**, C350-360. (2015).

94 Crump, J. A., Sjolund-Karlsson, M., Gordon, M. A. & Parry, C. M. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections. *Clin Microbiol Rev.* **28**, 901-937 (2015).

95 Barman, M. *et al.* Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infection and immunity* **76**, 907-915, doi:10.1128/IAI.01432-07 (2008).

96 Winter, S. E. *et al.* Gut inflammation provides a respiratory electron acceptor for Salmonella. *Nature* **467**, 426-429, doi:10.1038/nature09415 (2010).

97 Thiennimitr, P. *et al.* Intestinal inflammation allows Salmonella to use ethanolamine to compete with the microbiota. *Proc Natl Acad Sci U S A* **108**, 17480-17485, doi:10.1073/pnas.1107857108 (2011).

98 Niethammer, P., Grabher, C., Look, A. T. & Mitchison, T. J. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. *Nature* **459**, 996-999, doi:10.1038/nature08119 (2009).

99 Chang, S. *et al.* Dual oxidase regulates neutrophil recruitment in allergic airways. *Free Radic Biol Med* **65**, 38-46, doi:10.1016/j.freeradbiomed.2013.06.012 (2013).

100 Grasberger, H., El-Zaatari, M., Dang, D. T. & Merchant, J. L. Dual oxidases control release of hydrogen peroxide by the gastric epithelium to prevent Helicobacter felis infection and inflammation in mice. *Gastroenterology* **145**, 1045-1054, doi:10.1053/j.gastro.2013.07.011 (2013).

101 Pircalabioru, G. *et al.* Defensive Mutualism Rescues NADPH Oxidase Inactivation in Gut Infection. *Cell host & microbe* **19**, 651-663, doi:10.1016/j.chom.2016.04.007 (2016).

102 Walter, J., Britton, R. A. & Roos, S. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the Lactobacillus reuteri paradigm. *Proc Natl Acad Sci U S A* **108 Suppl 1**, 4645-4652, doi:10.1073/pnas.1000099107 (2011).

103 Alvarez, L. A. *et al.* NADPH oxidase-derived H2O2 subverts pathogen signaling by oxidative phosphotyrosine conversion to PB-DOPA. *Proc Natl Acad Sci U S A* **113**, 10406-10411, doi:10.1073/pnas.1605443113 (2016).

104 Fujii, N. & Saito, T. Homochirality and life. *Chem Rec* **4**, 267-278, doi:10.1002/tcr.20020 (2004).

105 Cava, F., de Pedro, M. A., Lam, H., Davis, B. M. & Waldor, M. K. Distinct pathways for modification of the bacterial cell wall by non-canonical D-amino acids. *EMBO J* **30**, 3442-3453, doi:10.1038/emboj.2011.246 (2011).

106 Sasabe, J. *et al.* Interplay between microbial d-amino acids and host d-amino acid oxidase modifies murine mucosal defence and gut microbiota. *Nature microbiology* **1**, 16125, doi:10.1038/nmicrobiol.2016.125 (2016).

107 Lambeth, J. D. & Neish, A. S. Nox enzymes and new thinking on reactive oxygen: a double-edged sword revisited. *Annu Rev Pathol* **9**, 119-145 (2014).

108 Jones, R. M. *et al.* Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J.* **32**, 3017-3028. (2013).

109 Jones, R. M. *et al.* Lactobacilli Modulate Epithelial Cytoprotection through the Nrf2 Pathway. *Cell Rep.* **12**, 1217-1225. (2015).

110 Alam, A. *et al.* Redox signaling regulates commensal-mediated mucosal homeostasis and restitution and requires formyl peptide receptor 1. *Mucosal Immunol* **7**, 645-655, doi:10.1038/mi.2013.84 (2014).

111 Babbin, B. A. *et al.* Formyl peptide receptor-1 activation enhances intestinal epithelial cell restitution through phosphatidylinositol 3-kinase-dependent activation of Rac1 and Cdc42. *J Immunol* **179**, 8112-8121 (2007).

112 Leoni, G. *et al.* Annexin A1, formyl peptide receptor, and NOX1 orchestrate epithelial repair. *J Clin Invest.* **123**, 443-454. (2013).

113 Mitra, S. K., Hanson, D. A. & Schlaepfer, D. D. Focal adhesion kinase: in command and control of cell motility. *Nat Rev Mol Cell Biol.* **6**, 56-68. (2005).

114 Alam, A. *et al.* The microenvironment of injured murine gut elicits a local pro-restitutive microbiota. *Nature Micro* **1**, In press (2016).

115 Chandel, N. S. & Schumacker, P. T. Cellular oxygen sensing by mitochondria: old questions, new insight. *J Appl Physiol (1985)* **88**, 1880-1889 (2000).

116 Hagen, T., Taylor, C. T., Lam, F. & Moncada, S. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. *Science* **302**, 1975-1978. (2003).

117 Schaible, B., Schaffer, K. & Taylor, C. T. Hypoxia, innate immunity and infection in the lung. *Respir Physiol Neurobiol.* **174**, 235-243. (2010).

118 Kelly, C. J. *et al.* Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe.* **17**, 662-671. (2015).

119 Glover, L. E., Lee, J. S. & Colgan, S. P. Oxygen metabolism and barrier regulation in the intestinal mucosa. *J Clin Invest.* **126**, 3680-3688. (2016).

120 Latz, E. NOX-free inflammasome activation. *Blood* **116**, 1393-1394, doi:10.1182/blood-2010-06-287342 (2010).

121 Karhausen, J. *et al.* Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. *J Clin Invest* **114**, 1098-1106 (2004).

122 Arteel, G. E., Thurman, R. G. & Raleigh, J. A. Reductive metabolism of the hypoxia marker pimonidazole is regulated by oxygen tension independent of the pyridine nucleotide redox state. *Eur J Biochem* **253**, 743-750 (1998).

123 Arteel, G. E., Thurman, R. G., Yates, J. M. & Raleigh, J. A. Evidence that hypoxia markers detect oxygen gradients in liver: pimonidazole and retrograde perfusion of rat liver. *Br J Cancer* **72**, 889-895 (1995).

124 Goethals, L. *et al.* Hypoxia in human colorectal adenocarcinoma: comparison between extrinsic and potential intrinsic hypoxia markers. *Int J Radiat Oncol Biol Phys* **65**, 246-254, doi:10.1016/j.ijrobp.2006.01.007 (2006).

125 Hindryckx, P. *et al.* Intrarectal administration of oxygenated perfluorodecalin promotes healing of murine colitis by targeting inflammatory hypoxia. *Lab Invest.* **91**, 1266-1276. (2011).

126 Niecknig, H. *et al.* Role of reactive oxygen species in the regulation of HIF-1 by prolyl hydroxylase 2 under mild hypoxia. *Free Radic Res* **46**, 705-717, doi:10.3109/10715762.2012.669041 (2012).

127 Synnestvedt, K. *et al.* Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J Clin Invest* **110**, 993-1002 (2002).

128 Cummins, E. P. *et al.* The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis. *Gastroenterology* **134**, 156-165. (2008).

129 Han, I. O., Kim, H. S., Kim, H. C., Joe, E. H. & Kim, W. K. Synergistic expression of inducible nitric oxide synthase by phorbol ester and interferon-gamma is mediated through NF-kappaB and ERK in microglial cells. *J Neurosci Res* **73**, 659-669. (2003).

130 Morote-Garcia, J. C., Rosenberger, P., Nivillac, N. M., Coe, I. R. & Eltzschig, H. K. Hypoxia-inducible factor-dependent repression of equilibrative nucleoside transporter 2 attenuates mucosal inflammation during intestinal hypoxia. *Gastroenterology* **136**, 607-618, doi:S0016-5085(08)01874-X [pii]
10.1053/j.gastro.2008.10.037 [doi] (2009).

131 Robinson, A. *et al.* Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. *Gastroenterology* **134**, 145-155. (2008).

132 Shah, Y. M. *et al.* Hypoxia-inducible factor augments experimental colitis through an MIF-dependent inflammatory signaling cascade. *Gastroenterology* **134**, 2036-2048, doi:S0016-5085(08)00440-X [pii]
10.1053/j.gastro.2008.03.009 [doi] (2008).

133 Giatromanolaki, A. *et al.* Hypoxia inducible factor 1alpha and 2alpha overexpression in inflammatory bowel disease. *J Clin Pathol* **56**, 209-213. (2003).

134 Mariani, F. *et al.* Cyclooxygenase-2 and Hypoxia-Inducible Factor-1alpha protein expression is related to inflammation, and up-regulated since the early steps of colorectal carcinogenesis. *Cancer Lett* **279**, 221-229, doi:S0304-3835(09)00085-8 [pii]

10.1016/j.canlet.2009.02.001 [doi] (2009).

135 Matthijsen, R. A. *et al.* Enterocyte shedding and epithelial lining repair following ischemia of the human small intestine attenuate inflammation. *PLoS One* **4**, e7045, doi:10.1371/journal.pone.0007045 [doi] (2009).

136 Colgan, S. P. & Taylor, C. T. Hypoxia: an alarm signal during intestinal inflammation. *Nat Rev Gastroenterol Hepatol* **7**, 281-287, doi:nrgastro.2010.39 [pii]

10.1038/nrgastro.2010.39 [doi] (2010).

137 Campbell, E. L. & Colgan, S. P. Neutrophils and inflammatory metabolism in antimicrobial functions of the mucosa. *J Leukoc Biol* **98**, 517-522, doi:10.1189/jlb.3MR1114-556R (2015).

138 Holden, V. I., Breen, P., Houle, S., Dozois, C. M. & Bachman, M. A. Klebsiella pneumoniae Siderophores Induce Inflammation, Bacterial Dissemination, and HIF-1alpha Stabilization during Pneumonia. *MBio* **7**, doi:10.1128/mBio.01397-16 (2016).

139 Kirienko, N. V. *et al.* Pseudomonas aeruginosa disrupts Caenorhabditis elegans iron homeostasis, causing a hypoxic response and death. *Cell host & microbe* **13**, 406-416, doi:10.1016/j.chom.2013.03.003 (2013).

140 Kelly, C. J. *et al.* Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell host & microbe* **17**, 662-671, doi:10.1016/j.chom.2015.03.005 (2015).

141 Donohoe, D. R., Wali, A., Brylawski, B. P. & Bultman, S. J. Microbial regulation of glucose metabolism and cell-cycle progression in mammalian colonocytes. *PLoS One* **7**, e46589, doi:10.1371/journal.pone.0046589 (2012).

142 Rivera-Chavez, F. *et al.* Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell host & microbe* **19**, 443-454, doi:10.1016/j.chom.2016.03.004 (2016).

143 Zambetti, L. P. & Mortellaro, A. NLRPs, microbiota, and gut homeostasis: unravelling the connection. *J Pathol.* **233**, 321-330. (2014).

144 Wen, H., Miao, E. A. & Ting, J. P. Mechanisms of NOD-like receptor-associated inflammasome activation. *Immunity* **39**, 432-441, doi:10.1016/j.immuni.2013.08.037 (2013).

145 Schroder, K. & Tschopp, J. The inflammasomes. *Cell* **140**, 821-832, doi:10.1016/j.cell.2010.01.040 (2010).

146 Coccia, M. *et al.* IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. *J Exp Med* **209**, 1595-1609, doi:10.1084/jem.20111453 (2012).

147 Neudecker, V. *et al.* Myeloid-derived miR-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. *J Exp Med* **214**, 1737-1752, doi:10.1084/jem.20160462 (2017).

148 Zaki, M. H. *et al.* The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* **32**, 379-391, doi:10.1016/j.immuni.2010.03.003 (2010).

149 Saitoh, T. *et al.* Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* **456**, 264-268, doi:10.1038/nature07383 (2008).

150 Dostert, C. *et al.* Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* **320**, 674-677, doi:10.1126/science.1156995 (2008).

151 Meissner, F. *et al.* Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. *Blood* **116**, 1570-1573, doi:10.1182/blood-2010-01-264218 (2010).

152 van de Veerdonk, F. L. *et al.* Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease. *Proc Natl Acad Sci U S A* **107**, 3030-3033, doi:10.1073/pnas.0914795107 (2010).

153 Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W. & Sheu, S. S. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *American journal of physiology. Cell physiology* **287**, C817-833, doi:10.1152/ajpcell.00139.2004 (2004).

154 Zhou, R., Yazdi, A. S., Menu, P. & Tschopp, J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**, 221-225, doi:10.1038/nature09663 (2011).

155 Birchenough, G. M., Johansson, M. E., Gustafsson, J. K., Bergstrom, J. H. & Hansson, G. C. New developments in goblet cell mucus secretion and function. *Mucosal Immunol* **8**, 712-719, doi:10.1038/mi.2015.32 (2015).

156 Chen, G. Y. & Stappenbeck, T. S. Mucus, it is not just a static barrier. *Sci Signal* **7**, pe11, doi:10.1126/scisignal.2005357 (2014).

157 Johansson, M. E. & Hansson, G. C. Is the intestinal goblet cell a major immune cell? *Cell host & microbe* **15**, 251-252, doi:10.1016/j.chom.2014.02.014 (2014).

158 Cadwell, K. *et al.* A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* **456**, 259-263, doi:10.1038/nature07416 (2008).

159 Patel, K. K. *et al.* Autophagy proteins control goblet cell function by potentiating reactive oxygen species production. *EMBO J* **32**, 3130-3144, doi:10.1038/emboj.2013.233 (2013).

160 Wlodarska, M. *et al.* NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell* **156**, 1045-1059, doi:10.1016/j.cell.2014.01.026 (2014).

161 Birchenough, G. M., Nystrom, E. E., Johansson, M. E. & Hansson, G. C. A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science* **352**, 1535-1542, doi:10.1126/science.aaf7419 (2016).

162 Sankey, E. A. *et al.* Early mucosal changes in Crohn's disease. *Gut* **34**, 375-381. (1993).

163 Nowarski, R. *et al.* Epithelial IL-18 Equilibrium Controls Barrier Function in Colitis. *Cell* **163**, 1444-1456, doi:10.1016/j.cell.2015.10.072 (2015).

164 Goto, Y. *et al.* Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science* **345**, 1254009, doi:10.1126/science.1254009 (2014).

165 Lindemans, C. A. *et al.* Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* **528**, 560-564, doi:10.1038/nature16460 (2015).

166 Zheng, Y. *et al.* Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med* **14**, 282-289, doi:10.1038/nm1720 (2008).

167 Eberl, G., Colonna, M., Di Santo, J. P. & McKenzie, A. N. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. *Science* **348**, aaa6566, doi:10.1126/science.aaa6566 (2015).

168 Munoz, M. *et al.* Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. *Immunity* **42**, 321-331, doi:10.1016/j.immuni.2015.01.011 (2015).

169 Bernink, J. H. *et al.* Interleukin-12 and -23 Control Plasticity of CD127(+) Group 1 and Group 3 Innate Lymphoid Cells in the Intestinal Lamina Propria. *Immunity* **43**, 146-160, doi:10.1016/j.immuni.2015.06.019 (2015).

170 Guan, Y., Worrell, R. T., Pritts, T. A. & Montrose, M. H. Intestinal ischemia-reperfusion injury: reversible and irreversible damage imaged in vivo. *Am J Physiol Gastrointest Liver Physiol* **297**, G187-196, doi:10.1152/ajpgi.90595.2008 (2009).

171 Wang, D., Mann, J. R. & DuBois, R. N. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. *Gastroenterology* **128**, 1445-1461 (2005).

172 Vane, J. R., Bakhle, Y. S. & Botting, R. M. Cyclooxygenases 1 and 2. *Annual review of pharmacology and toxicology* **38**, 97-120 (1998).

173 Moses, T., Wagner, L. & Fleming, S. D. TLR4-mediated Cox-2 expression increases intestinal ischemia/reperfusion-induced damage. *J Leukoc Biol* **86**, 971-980, doi:10.1189/jlb.0708396 (2009).

174 Blikslager, A. T., Roberts, M. C., Rhoads, J. M. & Argenzio, R. A. Prostaglandins I2 and E2 have a synergistic role in rescuing epithelial barrier function in porcine ileum. *J Clin Invest* **100**, 1928-1933, doi:10.1172/JCI119723 (1997).

175 Samuelsson, B. & Hammarstrom, S. Leukotrienes: a novel group of biologically active compounds. *Vitam Horm* **39**, 1-30. (1982).

176 Dennis, E. A. & Norris, P. C. Eicosanoid storm in infection and inflammation. *Nat Rev Immunol* **15**, 511-523 (2015).

177 Otamiri, T. Oxygen radicals, lipid peroxidation, and neutrophil infiltration after small-intestinal ischemia and reperfusion. *Surgery* **105**, 593-597 (1989).

178 Eltzschig, H. K., Bratton, D. L. & Colgan, S. P. Targeting hypoxia signalling for the treatment of ischaemic and inflammatory diseases. *Nat Rev Drug Discov.* **13**, 852-869. (2014).

179 Katada, K., Takagi, T., Uchiyama, K. & Naito, Y. Therapeutic roles of carbon monoxide in intestinal ischemia-reperfusion injury. *J Gastroenterol Hepatol.* **30**, 46-52. (2015).

180 Younes, M. *et al.* Oxidative tissue damage following regional intestinal ischemia and reperfusion in the cat. *Res Exp Med (Berl)* **184**, 259-264 (1984).

181 Parks, D. A., Williams, T. K. & Beckman, J. S. Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a reevaluation. *Am J Physiol* **254**, G768-774 (1988).

182 Harrison, R. Structure and function of xanthine oxidoreductase: where are we now? *Free Radic Biol Med* **33**, 774-797 (2002).

183 Eisen, A. *et al.* Ischemic preconditioning: nearly two decades of research. A comprehensive review. *Atherosclerosis* **172**, 201-210, doi:10.1016/S0021-9150(03)00238-7 (2004).

184 Alchera, E., Dal Ponte, C., Imarisio, C., Albano, E. & Carini, R. Molecular mechanisms of liver preconditioning. *World J Gastroenterol* **16**, 6058-6067 (2010).

185 Khoury, J., Ibla, J. C., Neish, A. S. & Colgan, S. P. Antiinflammatory adaptation to hypoxia through adenosine-mediated cullin-1 deneddylation. *J Clin Invest* **117**, 703-711. (2007).

186 Hatakeyama, S. *et al.* Ubiquitin-dependent degradation of IkappaBalpha is mediated by a ubiquitin ligase Skp1/Cul 1/F-box protein FWD1. *Proc Natl Acad Sci U S A* **96**, 3859-3863. (1999).

187 Boh, B. K., Smith, P. G. & Hagen, T. Neddylation-Induced Conformational Control Regulates Cullin RING Ligase Activity In Vivo. *J Mol Biol* **409**, 136-145. (2011).

188 Soucy, T. A. *et al.* An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732-736. (2009).

189 Ehrentraut, S. F. *et al.* Central role for endothelial human deneddylase-1/SENP8 in fine-tuning the vascular inflammatory response. *J. Immunol.* **190**, 392-400 (2013).

190 Eltzschig, H. K., Sitkovsky, M. V. & Robson, S. C. Purinergic signaling during inflammation. *N Engl J Med* **367**, 2322-2333, doi:10.1056/NEJMra1205750 (2012).

191 Eltzschig, H. K. *et al.* HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J Exp Med* **202**, 1493-1505. (2005).

192 Eltzschig, H. K. Adenosine: an old drug newly discovered. *Anesthesiology* **111**, 904-915, doi:10.1097/ALN.0b013e3181b060f2 (2009).

193 Xu, F. *et al.* Structure of an agonist-bound human A2A adenosine receptor. *Science.* **332**, 322-327. (2011).

194 Frick, J. S. *et al.* Contribution of adenosine A2B receptors to inflammatory parameters of experimental colitis. *J Immunol* **182**, 4957-4964 (2009).

195 Aherne, C. M. *et al.* Epithelial-specific A2B adenosine receptor signaling protects the colonic epithelial barrier during acute colitis. *Mucosal Immunol.* **8**, 1324-1338. (2015).

196 Hart, M. L. *et al.* Hypoxia-Inducible Factor-1alpha-Dependent Protection from Intestinal Ischemia/Reperfusion Injury Involves Ecto-5'-Nucleotidase (CD73) and the A2B Adenosine Receptor. *J Immunol*, doi:jimmunol.0903617 [pii]

10.4049/jimmunol.0903617 (2011).

197 Hart, M. L., Jacobi, B., Schittenhelm, J., Henn, M. & Eltzschig, H. K. Cutting Edge: A2B Adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury. *J Immunol* **182**, 3965-3968 (2009).

198 Eltzschig, H. K. *et al.* Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood* **104**, 3986-3992 (2004).

199 Elliott, M. R. *et al.* Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* **461**, 282-286 (2009).

200 Eltzschig, H. K. *et al.* ATP release from activated neutrophils occurs via connexin 43 and modulates adenosine-dependent endothelial cell function. *Circ Res* **99**, 1100-1108. (2006).

201 Eltzschig, H. K. *et al.* Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *The Journal of experimental medicine* **198**, 783-796, doi:10.1084/jem.20030891 (2003).

202 Weissmuller, T. *et al.* PMNs facilitate translocation of platelets across human and mouse epithelium and together alter fluid homeostasis via epithelial cell-expressed ecto-NTPDases. *J Clin Invest* **118**, 3682-3692 (2008).

203 Madara, J. L. *et al.* 5'-adenosine monophosphate is the neutrophil-derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers. *J. Clin. Invest.* **91**, 2320-2325 (1993).

204 Huang, C. *et al.* Genetic Risk for Inflammatory Bowel Disease Is a Determinant of Crohn's Disease Development in Chronic Granulomatous Disease. *Inflamm Bowel Dis.* **22**, 2794-2801. (2016).

205 Bao, S., Carr, E. D., Xu, Y. H. & Hunt, N. H. Gp91(phox) contributes to the development of experimental inflammatory bowel disease. *Immunol Cell Biol.* **89**, 853-860. (2011).

206 Strober, W., Fuss, I. J. & Blumberg, R. S. The immunology of mucosal models of inflammation. *Annu Rev Immunol* **20**, 495-549 (2002).

207 Abraham, C. & Cho, J. H. Inflammatory Bowel Disease. *N. Eng. J. Med.* **361**, 2066-2076 (2009).

208 Fournier, B. M. & Parkos, C. A. The role of neutrophils during intestinal inflammation. *Mucosal Immunol.* **5**, 354-366. (2012).

209 Schulzke, J. D. *et al.* Epithelial tight junctions in intestinal inflammation. *Ann N Y Acad Sci* **1165**, 294-300 (2009).

210 Butto, L. F. & Haller, D. Dysbiosis in intestinal inflammation: Cause or consequence. *Int J Med Microbiol.* **306**, 302-309. (2016).

211 Simpson, R. *et al.* Neutrophil and nonneutrophil-mediated injury in intestinal ischemia-reperfusion. *Ann Surg.* **218**, 444-453 (1993).

212 Kuhl, A. A. *et al.* Aggravation of different types of experimental colitis by depletion or adhesion blockade of neutrophils. *Gastroenterology* **133**, 1882-1892, doi:S0016-5085(07)01630-7 [pii]
10.1053/j.gastro.2007.08.073 [doi] (2007).

213 Wan, P. *et al.* Extracellular ATP mediates inflammatory responses in colitis via P2 x 7 receptor signaling. *Sci Rep* **6**, 19108, doi:10.1038/srep19108 (2016).

214 Wright, H. L., Moots, R. J., Bucknall, R. C. & Edwards, S. W. Neutrophil function in inflammation and inflammatory diseases. *Rheumatology (Oxford).* **49**, 1618-1631. (2010).

215 Tian, T., Wang, Z. & Zhang, J. Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. *Oxid Med Cell Longev* **2017**, 1-18 (2017).

216 Hebuterne, X., Filippi, J. & Schneider, S. M. Nutrition in adult patients with inflammatory bowel disease. *Curr Drug Targets* **15**, 1030-1038. (2014).

217 Tso, P., Lee, T. & Demichele, S. J. Lymphatic absorption of structured triglycerides vs. physical mix in a rat model of fat malabsorption. *Am J Physiol.* **277**, G333-340. (1999).

218 Hering, N. A., Fromm, M. & Schulzke, J. D. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J Physiol.* **590**, 1035-1044. (2012).

219 Capaldo, C. T. & Nusrat, A. Cytokine regulation of tight junctions. *Biochim Biophys Acta.* **1788**, 864-871. (2009).

220 Capaldo, C. T. & Nusrat, A. Claudin switching: Physiological plasticity of the Tight Junction. *Semin Cell Dev Biol.* **42**, 22-29 (2015).

221 Tolstanova, G. *et al.* Early endothelial damage and increased colonic vascular permeability in the development of experimental ulcerative colitis in rats and mice. *Lab Invest.* **92**, 9-21. (2012).

222 Brown, I. A., McClain, J. L., Watson, R. E., Patel, B. A. & Gulbransen, B. D. Enteric glia mediate neuron death in colitis through purinergic pathways that require connexin-43 and nitric oxide. *Cell Mol Gastroenterol Hepatol.* **2**, 77-91. (2016).

223 Scirocco, A. *et al.* Exposure of Toll-like receptors 4 to bacterial lipopolysaccharide (LPS) impairs human colonic smooth muscle cell function. *J Cell Physiol* **223**, 442-450, doi:10.1002/jcp.22053 (2010).